Cloacal Glands of the Group-Living Lizard, *Cordylus cataphractus* (Sauria: Cordylidae)

by

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- DECLARATION -

I the undersigned, hereby declare that the work contained in this thesis is my own original work and has not previously in its entirety or in part been submitted at any university for a degree.

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Signature **Date**

- ABSTRACT -

Standard histological procedures revealed that there are two types of glands in the cloacal complex of the viviparous *Cordylus cataphractus*. These occur in the urodeal region of the complex (called urodeal glands), and in the posterior proctodeum region (called proctodeal glands). Urodeal glands are found in females only and differentiate at sexual maturity. Proctodeal glands occur in both sexes and the dorsal and ventral proctodeal glands are identical in structure. Seasonal activity in cloacal gland structure and secretory was studied. Urodeal glands show distinct seasonal variation in development and secretory activity, they become enlarged in vitellogenic females, remain active during pregnancy, with glandular activity peaking around parturition. Proctodeal glands, in contrast, show little or no seasonal variation in development or secretory activity in both sexes. Secretion quantity, however, is highly variable among individuals of the same sex, time of year, as well as reproductive stage, but evidently much less in females than in males. Although the possible functions of the cloacal glands are still unclear, two main functions are suggested: a) mechanical function such as lubrication or a substrate for genital products and b) pheromonal communication. I propose that urodeal glands are involved in some reproductive function rather than in social communication, because very little secretion is found during the mating season and peak glandular activity are evident around parturition. The opposite may be true for proctodeal glands, but further investigation is needed, particularly chemical analysis of secretion and behavioural manipulation, to determine the role of these glands in social communication.

- UITTREKSEL -

Standaard histologiese tegnieke dui aan dat daar twee tipes kliere voorkom in die kloakale kompleks van die lewendbarende akkedis, *Cordylus cataphractus*. Die een kliertipe word in die urodeale omgewing aangetref en staan bekend as die urodeaalkliere, terwyl die ander groep in die posterior area van die proktodeum voorkom en proktodeaalkliere heet. Urodeaalkliere word slegs in wyfies aangetref en ontwikkel eers na volwassenheid bereik is. Proktodeaalkliere kom in albei geslagte voor en dorsale en ventrale aspekte van hierdie kliere is identies aan mekaar. Die seisoenale aktiwiteit in struktuur en sekresie-aktiwiteit van hierdie kloakale kliere is ondersoek in mannetjies en wyfies wat gedurende verskillende maande van die jaar versamel is en dus in verskillende voorplantingstadia was. Urodeaalkliere het duidelike seisoenale variasie vertoon wat beide ontwikkeling van die klier en sekretoriese aktiwiteite betref. Die klier vergroot tydens vitellogenese, bly aktief tydens swangerskap en bereik maksimale aktiwiteitsvlakke rondom geboorte. In teenstelling hiermee het die proktodeaalkliere van beide geslagte weinig of self geen seisonale variasie in hul ontwikkeling of sekretoriese aktiwiteit getoon nie. Die hoeveelheid sekreet vrygestel was merkbaar minder in wyfies as in mannetjies, maar hoogs veranderlik tussen individue van dieselfde geslag of voortplantingstadium; of wat in dieselfde tydperk van die jaar versamel is. Alhoewel funksies van kloakale kliere steeds onduidelik bly, word twee hooffunksies voorgestel: a) ‘n meganiese funksie, byvoorbeeld as smeermiddel of as ‘n substraat vir geslagsprodukte en b) ‘n kommunikasie funksie, byvoorbeeld in die vrystelling van feromone. Ek stel

voor dat die urodeaalkliere eerder by ‘n voortplantingsfunksie betrokke is, aangesien baie min sekreet gedurende die paarseisoen vervaardig word terwyl klieraktiwiteit rondom geboorte ‘n piek bereik. Die teenoorgestelde mag geld vir proktodeaalkliere, maar vereis verdere ondersoek. Veral ‘n chemiese analise van die sekresie en gedragsmanipulasie sal nuttig wees om ‘n moontlike rol van hierdie kliere in sosiale kommunikasie te bepaal.

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CHAPTER 1: GENERAL INTRODUCTION

1. Basic topography and functions of the tetrapod cloaca

Standard textbooks on vertebrate morphology (e.g., Hildebrand & Goslow, 2001; Kent & Carr, 2001; Kardong, 2002) describe the tetrapod cloaca (present in reptiles, birds and monotremes) as a common receiving chamber for intestinal, kidney and gonadal products, structurally influenced by three functions, namely defecation, urination and copulation. Its compartmentalized design, brought to attention by Hans Gadow during the late nineteenth century, supports this simplistic view. The most proximal compartment is the coprodeum into which the intestines open, while urinary and genital ducts open into the intermediate urodeum, and the most distal compartment, the proctodeum, often develops a penis. These named functions may be fundamental to the cloaca, but certainly not the only ones.

The tetrapod cloaca is not merely a passageway - it may be involved in modifying some of mentioned products (e.g., in fecel pellet formation) and it adds secretions to some of these (e.g., in spermatophore formation), mostly for lubrication or mechanical purposes. The cloaca may also be actively involved in osmoregulation (by storing or altering urine itself, or passing it to the urinary bladder or colon for storage or alteration). The female cloaca may store spermatozoa after copulation. Also, a large literature reports on a function not necessarily related to passage of urogenital and alimentary products, namely that of chemical communication. Regarding the latter, and also for lubrication,

cloacal glands have often been implicated to play the principal part (Cooper *et al.*, 1986; Trauth *et al.*, 1987).

Much is known about the gross anatomy of the tetrapod cloaca, but the functional significance of the cloacal glands is still not well understood. This is because there is a general paucity in detailed histological, histochemical and experimental data pertaining to occurrence, secretory activity, and nature of secretions of these glands. Kardong (2002) provides a brief, but efficient, summary of the topography of reptilian cloacae - I will therefore focus on summarising available information on cloacal gland occurrence and functions in the major tetrapod groups in this chapter.

2. Cloacal gland function in major tetrapod groups

Cloacal anatomy has been particularly well studied in anurans (Heidenhain, 1889; Dawson, 1922; Francis, 1934; Van Dijk, 1955) and salamanders (Brizzi *et al.*, 1989; Sever, 1978, 1981, 1983, 1985, 1987, 1988a,b, 1991, 1992, 1995, Sever *et al.*, 1990a, b; Verrel & Sever, 1988; Williams *et al.*, 1984, 1985). These reports illustrate a great diversity in cloacal topography and cloacal gland organisation even among closely related taxa. Cloacal anatomy has therefore been readily used for taxonomic inferences in amphibians (see Sever, 1991). Little is known about cloacal gland function in amphibians, however, most suggestions in the literature are purely speculative, although some assumptions have been based on histological interpretations (see Sever, 1988a, b; Sever *et al.*, 1990). In male salamanders, five types of cloacal glands are recognized, depending on their position and structure. All of these glands do not necessarily occur in

all species, however (Sever, 1991). Females apparently possess only ventral glands (Guillaume, 2000) and salamander spermathecae (Sever, 1994) in the cloacal region. In males, the dorsal glands are generally believed to secrete courtship pheromones and play a role in spermatophore production and females store spermatozoa in the glandular spermathecae (Sever, 1991, 1994). Kikuyama *et al.* (1997) found that cloacal glands of the male Red-bellied Newt, *Cynops pyrrhogaster*, are the source of a female-attracting peptide pheromone, namely Sodefrin.

The basic composition of the reptilian cloaca was brought to attention by a comparative study of the reproductive systems of vertebrates by Lereboullet (1851) and the work of Gadow (1887). Gabe & St Girons (1965) contributed importantly to comprehensive information about the anatomy, histology and histochemistry of the cloacae of almost 60 species of reptiles, while Whiting (1969) reported on more than 80 species. These studies, as well as more recent ones reporting on reptilian cloacae, focused almost exclusively on squamates (i.e. lizards, snakes and amphisbaenians). I will summarize details on cloacal gland function of squamates under a separate heading later in this chapter, as it has more direct relevance to the present study. Very little data exist on cloacal gland function in the other major reptilian groups, the rhyncocephalians, crocodilians and chelonians.

Cloacal glands of *Sphenodon punctatus* have been described by Gabe & Saint Girons (1965) as large paired masses, located ventro-lateral to the cloaca. Luppa (1977) mentioned briefly that cloacal glands have a simple tubular structure in *Sphenodon* and the function(s) of these glands remain unknown in rhyncocephalians.

It has long been believed that cloacal glands are absent from the cloacal region of chelonians, but this may be due to inadequate histological examination of this region, few studies thus refer to the presence of glands in these animals. Some chelonians discharge faeces and repellent secretions as a defensive mechanism when threatened - the secretions are apparently products of cloacal glands (http://reptilis.net/chelonia/senses.html). It has been mentioned in an information document by the Arizona-Sonora desert Museum in 1998 (www.desertmuseum.org/ programs/ exh_tortoiseinfo.html) that the desert tortoise (*Gopherus agassizii*) may use cloacal gland secretions for territorial marking.

Paired masses of tubulous scent glands have been observed in the lateral walls of different crocodilians (Rathke, 1866; Disselhorst, 1904; Gerhardt, 1933). Kuchel & Franklin (1998) suggested that the cloacal complex of crocodiles is important for osmoregulation. The coprodeum produces mucus to assist in the dehydration of faeces; and it may also play a role in fecal lubrication. The urodeum apparently functions as a primary site for post-renal modification and storage of urine, as no urinary bladder is found in crocodiles (Kuchel & Franklin, 2000).

Bird cloacae and their glands have practically being ignored until the 1960's (Whiting, 1969), and studies since have almost exclusively focused on the cloacal anatomy of the economically important domestic chicken, *Gallus gallus* (Lake *et al*., 1985; Etches, 1996; Elbrond *et al*.; 1997), and on various aspects of the foam gland in Japanese quail, *Coturnix japonica* (Coil & Wetherbee, 1959; Nagra, 1959; Klemm, 1975; Quay, 1967; Sachs, 1967; Massa *et al.*, 1980; Delville *et al.*, 1984; Mohan *et al*., 2001). The male quail produce large amounts of foam that is transferred to the female during

copulation, enhancing fertilization success. The source is the foam gland complex, a large, sexually dimorphic, external protuberance of the dorsal cloaca, consisting of glandular units between the striated muscle fibers of the cloaca sphincter muscles (Seiwert & Adkins-Regan, 1998). The cloacal gland in quails is androgen-dependent and positively correlated with testes size as well as sexual activity, therefore the size of the cloacal gland or frequency of foam discharge from this gland can be used as an indirect tool to predict the fertilization ability of particular males (Mohan *et al*., 2001).

The monotremes, considered to represent the earliest stage of mammal evolution, have captured the attention of naturalists since the 1800's, but little information exists about their cloacae and cloacal glands. Burrell (1927) described that, during egg-laying in *Platypus*, the eggs are coated with a sticky secretion from the oviduct walls at the moment of extrusion so they can adhere to one another. He did not mention the presence of any cloacal glands, however.

3. Cloacal gland presence and functions in squamates

Gadow (1887) mentioned that the snake cloacal complex is similar in structure to that of lizards and that well-developed glands are present lateral to the proctodeum. Several authors over the years have made broad statements about the cloacal gland structure in snakes without referring to specific species (Beuchelt, 1936; Whiting, 1969). Gabe & Saint Girons (1965) stated that there are dorsal glands in some snakes, but no ventral ones. The only accounts on the presence of ventral glands in snakes are those of Disselhorst (1904) and Beuchelt (1936). In addition to proctodeal glands, scent glands

have also been described to be present in snakes. These occur at the base of the tail and open through two ducts at the margin of the cloaca (Gadow, 1887; Gabe & Saint Girons, 1965; Whiting, 1969). The histology of cloacal scent glands was examined by Young *et al*. (1999) in about 50 species of snakes, but no apparent relationship between phylogeny and the morphological variation was found. The malodorous secretions from these glands, which typically are discharged when snakes are disturbed, generally are thought to repel predators, but the secretions may contain pheromones used in sex or species recognition as well (Weldon & Leto, 1995).

In amphisbaenians, cloacae have four different types of glands, i.e., precloacal-, dorsal-, ventral (or mentral) glands, and a gland at the apex of the hemipenis. Functions of these glands are unknown (Whiting, 1967).

Two populations of compound exocrine glands are known to occur within the three-chambered cloacae of lizards - one is associated with the intermediate urodeum (urodeal glands) and the other with the posterior proctodeum (proctodeal glands); the anterior coprodeum houses none. Although a number of studies report the histology of these glands in lizards (see Appendix 1), little is known about their function. Two broad functions have been proposed, often unsubstantiated by chemical or experimental evidence, namely a mechanical function, and that of pheromonal communication.

a) Mechanical function

Mechanically, cloacal gland secretions may simply facilitate the moment of mating or serve as substrate lubrication for passage of fecel and/or reproductive products (e.g., fecel pellets, eggs/young, etc.) as has been proposed by numerous authors, including

Lerebouillet (1851), Regamey (1935), Van der Merwe (1944), and Gabe & Saint Girons (1965). Wöpke (1931) pointed out the possibility that cloacal gland secretions may displace sperm towards the female genitals. Another possibility is that secretions may be added during spermatheca formation, much as is found in salamanders (see Sever, 1986, 1991). It may also act as a barrier (physical, and chemical if it has antibacterial properties) to protect the oviducts and their contents.

b) Pheromonal communication

Pheromones are apparently critical in stimulating copulation in snakes, but it is not clear how important they are in lizards (Cooper *et al*., 1986). Secretions from cloacal glands have also been related to mate attraction in snakes, particularly in female pythons, which lay scent trails to attract males (Withers & O'Shea, 1993).

Simon (1983) listed that lizards may use chemical cues for locating prey, detecting predators, identifying conspecifics, for sex discrimination, identification of eggs, aggregation, territorial marking and related spacing behaviour, and for exploratory behaviour.

Recent experimental and behavioural studies in mainly scincomorphs, revealed that many lizard species use chemical signals for communication. Cooper & Trauth (1992) suggested that *Gerrhosaurus nigrolineatus* and other cordyliforms might employ pheromonal communication as well, possibly in a manner similar to that of the scincid, *Eumeces laticeps.*

Studies by Cooper & Vitt (1984a, 1986a,b) and Cooper & Gartska, (1987a) revealed that males of the species *Eumeces laticeps* can discriminate between chemical

cloacal stimuli of male and female conspecifics and heterospecifics. Studies by Cooper & Vitt (1986), Cooper & Gartska (1987a), Trauth *et al.* (1987), Cooper & Trauth (1992), and Cooper *et al*. (1996) indicated that the proctodeal glands of the Scincidae and Gerrhosauridae must be considered a potential source of species-identifying pheromones present in both sexes. Bull *et al.* (1999) established that the scat-piling skink, *Egernia striolata*, could discriminate between its own scat and that of conspecifics, suggesting that the lizard respond to epidermal or cloacal gland secretions. It is possible that scats produced in the hindgut could become coated with a secretion from cloacal glands as they pass out of the cloaca.

Both broad functions suggested for urodeal and proctodeal glands in lizards (i.e., mechanical and chemical communication) have to do with at least some aspects of reproduction. Possible cloacal gland functions can thus be determined by studying seasonal variation in secretory activity and the nature of the secretions, especially if data on ecology, reproduction and sociality of the species are available. I have studied seasonal variation in size and microscopic anatomy of urodeal and proctodeal glands in a viviparous lizard, *Cordylus cataphractus*, as well as the nature of their secretion through histochemical techniques, in an attempt to infer the functional significance of these glands.

4. The study animal

The Armadillo Lizard, *Cordylus cataphractus* (Fig. 1) belongs to the Cordylidae family, which is endemic to sub-Saharan Africa and has a distinct clustering of species in

the south (Mouton & Van Wyk, 1997). Cordylids are typical sit-and-wait foragers, lacking the ability to discriminate prey chemicals by means of tongue-flicking behaviour (Branch, 1988a; Cooper *et al.*, 1997). The more than 50 species in this small family have previously been divided into four nominal genera (Loveridge, 1944; Branch, 1998), namely *Platysaurus* (flat lizards), *Cordylus* (girdled lizards), *Pseudocordylus* (crag lizards), and *Chamaesaura* (grass lizards). Lang (1991) established, based on morphological characters, that the genus *Chamaesaura* is the earliest diverging taxon, while *Cordylus* is the sister taxon of a *Pseudocordylus-Platysaurus* clade. Studies by Herselman *et al.* (1992) and Mouton & van Wyk (1997), however, showed that *Cordylus* and *Pseudocordylus* are paraphyletic. Herselman *et al.* (1992) divided the family into two subfamilies, the monogeneric Chamaesaurinae (*Chamaesaura*) and Cordylinae (*Platysaurus*, *Cordylus* and *Pseudocordylus*). Mouton & van Wyk (1997) proposed in their overview that Cordylidae is composed of two monophyletic groups, a *Platysaurus* clade and a *Cordylus-Pseudocordylus-Chamaesaura* clade with *Platysaurus* the most basal genus in the family. Frost *et al.* (2001) recently incorporated results of molecular analyses into the existing (mainly morphological) character database of Lang (1991) and Harvey & Gutberlet (1995). They suggested that *Platysaurus* is the sister taxon of all remaining cordylids and that both *Chamaesaura* and *Pseudocordylus* be transferred to the genus *Cordylus* to render a monophyletic taxonomy. Importantly, all members of *Platysaurus* are oviparous and those of *Cordylus* are viviparous. Understanding of the cordylid phylogeny is still incomplete and the relationships within the Cordylidae family are still taken as unresolved, even though its near outgroups and monophyly have been

known for a significant length of time (McDowell & Bogert, 1954; Estes *et al.*, 1988; Frost *et al.*, 2001). Characters of the cloacal complex were not used in previous (morphological) cladistic analyses, because very little information was available for any of the species.

Cordylus cataphractus is a group-living lizard, endemic to the west coast of southern Africa. Some data exist on its general ecology, (e.g. Mouton *et al.*, 1999), foraging ecology (e.g. Mouton *et al.*, 2000b), taxonomy (e.g., FitzSimons, 1943; Loveridge, 1944; Broadley, 1978; Lang, 1991; Frost *et al.*, 2001), behavioural ecology and sexual dimorphism (e.g., FitzSimons, 1943; Lang, 1991; Mouton *et al*., 1999; Visagie, 2001), as well as reproduction (Flemming & Mouton, 2002). The lizard is probably best known for the distinctive anti-predatory behaviour to which it owes its common name, the Armadillo Girdled Lizard. The lizard exhibits the peculiar habit of gripping its tail firmly in the mouth and rolling into a tight ball when threatened (Peers, 1930; FitzSimons, 1943; Branch, 1998; Mouton *et al.*, 1999). It will maintain this position as long as danger threatens. Retief (2000) found that significantly higher numbers of generation glands occur in low-density populations of this species, compared to high-density populations. Communication dynamics may thus differ in sparse versus dense populations of this species.

The selection of *C. cataphractus* as study animal was based on the availability of sufficient material in museum collections, as well as the availability of the above information on *C. cataphractus*. Also, this species lives permanently in groups, which is an uncommon phenomenon among lizards, and which may have an influence on

communication in the populations. By relating the above information to glandular activity, it may be possible to determine the functional significance of these cloacal glands. It will also allow for comparison of oviparous versus viviparous species as well as solitary-living versus group-living ones within the Cordyliformes.

The functions of cloacal glands in lizards are often proposed to relate to reproductive activities, I thus consider it relevant to briefly outline the reproductive cycles of male and female *C. cataphractus* in this chapter. In a study by Flemming & Mouton (2002), it was found that although *C. cataphractus* conforms to the basic pattern in reproductive cyclicity reported for cordylids (see review of Van Wyk & Mouton, (1998)), stages of reproduction, such as ovulation, mating and parturition, usually occur later than in the other species. *Cordylus cataphractus* females exhibit late autumn to spring vitellogenesis while activity in other cordylids is autumn to winter. Ovulation occurs during late spring (other cordylids: late winter to early spring) and parturition in early to mid-autumn (other cordylids: mid- to late autumn). Mating usually takes place in late spring (other cordylids: early to mid-spring) and *C. cataphractus* males and females have synchronous reproductive cycles. Males follow a prenuptual spermatogenic cycle, with peak spermatogenesis coinciding with spring ovulation in females. Most males have testes in a regressed state during November to April (summer and autumn).

As already mentioned, different lines of evidence may be used for determining cloacal gland function. In the present study I have employed three levels of investigation. First, I describe the gross and histological anatomy of the cloaca and its glands through dissection and histology. Then I report seasonal variation in glandular histology and

lastly, I give details on different histological staining techniques to deduce the broad chemical nature of secretions by these glands.

The present study is a product of the ongoing research done by the Cordylidae Research Team, which forms part of the Vertebrate Functional Biology Group, based at Stellenbosch University. It forms part of a long-term project of which the goal is to conduct a phylogenetic analysis of glands and social structures within the Cordylidae family.

This thesis includes four chapters, each prepared as a separate article. Repetition, especially in the introductory paragraphs, was therefore unavoidable. A general introduction (Chapter 1) provides background information on the anatomy of the tetrapod cloacal complex, cloacal glands in general and the family Cordylidae as well as a background of the study animal, *Cordylus cataphractus*, in order to avoid unnecessary repetitions in the introductions of the subsequent three chapters. Chapter 2 presents a general description of the anatomy of the cloacal complex of *C. cataphractus*. Chapters 3 and 4 are concerned with histological and histochemical investigations of cloacal glands (urodeal glands in Chapter 3 and proctodeal glands in Chapter 4) present in this lizard.

Figure 1. Photo of an Armadillo Girdled Lizard, *Cordylus cataphractus*.

CHAPTER 2: CLOACAL ANATOMY OF THE ARMADILLO LIZARD, *CORDYLUS CATAPHRACTUS*

- INTRODUCTION -

The cloaca in lizards, snakes and amphisbaenians (squamates) is a common chamber at the terminal part of the lower intestinum, which receives, relays, and modifies urogenital products. It opens to the outside through a cloacal opening, which in these animals is a transverse slit at the ventral base of the tail, usually covered by a posteriorly projecting shield of scales (Withers & O'Shea, 1993). Gadow (1887) devided the tetrapod cloacal complex into three compartments, namely the anterior coprodeum, the intermediate urodeum, and the posterior proctodeum. All three compartments are present in squamates, but the proctodeum is usually reduced compared to that of other tetrapods (see Kardong (2002) for a brief overview of basic cloacal topography among tetrapods).

In lizards, a rectocoprodeal fold (usually provided with a sphincter muscle) delineates the boundary between the posterior end of the intestine and the coprodeal depression (Hardy & Cole, 1981; Kardong, 2002). This boundary is not always well defined (usually in males), as the rectocoprodeal fold may be low and a circular muscle arrangement inconspicuous. In such cases, the change in nature of the mucosa may be helpful for determining the boundary. In Cordyliformes, for instance, the rectum has goblet cells, but the coprodeum lacks them (Mackay, 1993). Likewise, the boundary

between the coprodeum and urodeum is usually not well delimited in lizards and a common cavity between the coprodeum and urodeum (which may be called ‘copro-urodeum’) forms before the junction with the proctodeum. A conspicuous uroproctodeal fold and sphincter muscle usually separates the urodeum from the proctodeum (Whiting, 1969; Hardy & Cole, 1981).

There are distinct differences in cloacal morphology between the sexes in numerous lizards (Gabe & Saint Girons, 1965; Whiting, 1969; Hardy & Cole, 1981; Trauth *et al*., 1987; Mackay, 1993). The female urodeum, for instance, is more complex than in males, with prominent paired cylindrical dorsal limbs present and tall epithelial folds dominating the dorsal walls (Gabe & Saint Girons, 1965). Distinct urodeal glands usually occur in the submucosa of each urodeal limb in adult females. The urogenital ducts open separately into the urodeum, the oviducts more anteriorly into the urodeal limbs and ureters more posteriorly into the common urodeum. Males do not possess urodeal limbs or glands, and the mucosal folds of the urodeum are small or absent (Gabe & Saint Girons, 1965; Whiting, 1969; Hardy & Cole, 1981; Trauth *et al*., 1987; Mackay, 1993). The urogenital ducts in males open conjointly into the urodeum, usually through urogenital papillae (Whiting, 1969; Trauth *et al*., 1987; Mackay, 1993). The latter being lateral diverticula from the posterior portion of the urodeum. An urinary bladder extends ventrally from the urodeum in both sexes and is connected to it by means of a so-called bladder stalk. The proctodeum, housing hemipenes in males, is usually short and in both sexes surrounded by clusters of proctodeal glands (Gabe & Saint Girons, 1965; Whiting, 1969; Hardy & Cole, 1981; Trauth *et al*., 1987; Mackay, 1993). Proctodeal glands in

lizards, often also called dorsal and/or ventral glands, may differ among sexes in size and secretory activity (Cooper & Trauth, 1992).

Little is known about the functions of urodeal and proctodeal glands in lizards. Two main functions are often assumed, but rarely substantiated by chemical or experimental evidence, namely a mechanical function and that of pheromone production. Mechanically, secretions from these glands may displace sperm towards female genitals, facilitate the moment of mating, or serve as substrate lubrication for fecel and/or reproductive products (Lerebouillet, 1851; Wöpke, 1931; Regamey, 1935; Van der Merwe, 1944; Gabe & Saint Girons, 1965). Secretions from these glands may additionally or alternatively carry pheromones, which could be used for communication in locating prey, detecting predators, identifying conspecifics, sex discrimination, aggregation behaviour, territorial marking or in reproductive signals (Cooper & Vitt, 1984b, 1986a,b, 1987; Cooper *et al.*, 1986; Cooper & Gartska, 1987a; Trauth *et al.*, 1987; Cooper, 1992; Cooper & Trauth, 1992; Bull *et al.*, 1999).

Detailed histological data on cloacae and their glands are available for representatives of relatively few lizards of the families Lacertidae (Gadow, 1887; Wöpke, 1931; Regamey, 1935; Ihle, 1947), Teiidae (Hardy & Cole, 1981), Scincidae (Trauth *et al.*, 1987), Gerrhosauridae (Cooper & Trauth, 1992) and Cordylidae (Mackay, 1993). A functional and phylogenetic perspective of cloacal morphology in lizards seems compromised of so few detailed studies, especially in the light of the great variation in topography and histology obvious from these studies. Future investigations into lizard

cloacal morphology and function should include representatives of more families, also of more members from the same family.

The present study reports details on cloacal topography and histology in a cordylid species, the Armadillo Lizard, *Cordylus cataphractus.* Two congenerics, *C. cordylus* and *C. melanotus,* have been studied previously by Mackay (1993). All three species belong to a viviparous clade within Cordylidae, and *C. cataphractus* has a relatively basal position in this clade (Frost *et al*., 2001). The family has another clade of oviparous members, formally recognised as the genus *Platysaurus.* A single origin of viviparity is thus assumed within the Cordylidae (Blackburn, 1985, 1999). Members of the sister family, the Gerrhosauridae, are all oviparous and detailed histological data are available for a single species, namely *Gerrhosaurus nigrolineatus* (Cooper & Trauth, 1992). Mackay (1993) noted only minor differences in cloacal topography and histology between the *C. cordylus* and *C. melanotus*. Differences between the gerrhosaurid species studied by Cooper & Trauth (1992) and the two species of *Cordylus* investigated by Mackay (1993) appear to be more considerable. Mackay (1993) postulated that these differences might be related to differences in parity mode.

Cordylus cataphractus is like most other cordylids rock-dwelling and exhibits a sit-and-wait foraging strategy (Mouton, *et al.*, 1999). Unlike the two species of *Cordylus* studied by Mackay (1993), and most other cordylids for that matter, *Cordylus cataphractus* is not a solitary-living form (Peers, 1930; Branch, 1998; Mouton *et al.*, 1999; Visagie, 2001). It displays permanent grouping behaviour and there could thus be differences in communication dynamics when compared to congenerics (Visagie, 2001).

If cloacal gland secretions are involved in pheromonal communication in *Cordylus*, then these glands may differ in structure, secretory activity and nature of secretions when *C. cataphractus* is compared with solitary-living congenerics.

The aims of the present study are to a) describe cloacal topography, including position of entry of urogenital ducts, in both sexes of *C. cataphractus*; b) to describe the histological appearance of the walls, including the glands found therein, of the different cloacal compartments; c) and to describe the histological appearance of urogenital ducts at the site of entering those compartments. The mentioned descriptions will hopefully provide some answers to the following questions: How great are the structural differences in cloacal anatomy, if any, among members of *Cordylus*? Are such differences related to sex? Are such differences related to life-style (i.e., the solitary-living gerrhosaurid versus group-living cordylid)? Are structural differences related to parity mode (i.e., the viviparous cordylid versus the oviparous gerrhosaurid)?

- MATERIALS AND METHODS -

1. *Materials*

Cordylus cataphractus specimens were collected along the lowland plains of the west coast of South Africa, between 1996 and 2000. Collection was restricted to a relatively small area of approximately 120 km^2 in the Lamberts Bay-Graafwater district (ca 31°55'S 18°24'E), to control for geographic variation in the parameters investigated. Individuals of this species on the coastal plains attain a larger average snout-vent length, possess less generation glands, and have darker coloration than lizards of the higher altitudes (Visagie, 2001).

Lizards were sedated and killed on the day of capture by injecting an overdose of sodium pentobarbitone into the abdominal cavity. Specimens were then fixed in 10 % formalin before being transferred to 70 % ethyl alcohol. All specimens were deposited into the Ellerman Collection at Stellenbosch University. The snout-vent length (SVL) of all specimens was recorded to the nearest 0.1 mm using digital calipers. Size at sexual maturity for both sexes was taken as 95 mm SVL (Flemming & Mouton, 2002). Males were distinguished from females by the presence of conspicuous hemipenal bulges, broad heads, and the presence of a relative large number of generation glands (Van Wyk & Mouton, 1992).

Cloacal complexes of one male specimen (USEC/H-3479) and one female (USEC/H-3480) were excised, dissected and examined by stereomicroscope to describe the gross anatomy thereof. Cloacae of a further three specimens of each sex were subjected to histological procedures and examination by light microscope. Specimens were selected to represent at least a non-reproductive and reproductively active stage of each sex (Table 1). See Appendix 2 for a list of all specimens used, with details on collection- and locality data. Generalized diagrams of cloacal topographies were drawn for both sexes, after dissection and examination by stereomicroscope (see Figs. 2 & 3).

2. Histological preparation of tissue samples

Entire cloacal complexes along with portions of the gonads, urogenital ducts, kidneys, and rectum were excised from the specimens, dissected to remove attached mesenteries, blood vessels and muscles, and placed in 70 % ethanol until further histological processing (Preece, 1972; Humason, 1997). Tissues were dehydrated in increasing concentrations of ethanol, cleared in toluene and embedded in paraffin wax (58°C melting point). Cloacae were serially sectioned at a thickness of 6 - 11 µm. All cloacae were cut transversally. Slides were stained with Erlich's Haematoxylin and Eosin (H & E) for general cytological examination. Photographs were taken using digital cameras fixed to stereo and compound microscopes and Leica Quips Image Analysing Software. Cloacal terminology generally follows Whiting (1969), Hardy & Cole (1981) and Mackay (1993).

Table 1. *Cordylus cataphractus* specimens used for gross and histological morphological examination of their cloacae.

Reproductive Status	Date Collected	Lodging code	Sex	SVL (mm)
Inactive	1996/12/05	USEC/H-3099	Adult male	115.3
Early spermatogenic	1996/10/24	USEC/H-3070	Adult male	108.7
Late spermatogenic	1996/10/24	USEC/H-3080	Adult male	119.7
Pre-vitellogenic	1997/04/02	USEC/H-3189	Adult female	98.59
Late vitellogenic	1996/10/24	USEC/H-3085	Adult female	110.58
Gravid	1997/04/02	USEC/H-3149	Adult female	114.0

- RESULTS -

1. Gross anatomy of the cloacal complex

Generalized drawings of cloacal complexes of male and female *Cordylus cataphractus* are presented in Figs. 2 & 3. The three subdivisions of lizard cloacae, originally described by Gadow (1887), namely the coprodeum, urodeum and proctodeum, could easily be distinguished in *C. cataphractus*. The coprodeum (CO) follows the posterior portion of the large intestine (IN), with the relatively short, dorsally extending urodeum (UD) after the coprodeum. The proctodeum (PR) is a short tube ending in the transverse vent. The paired elongated, kidneys or metanephri (MN) are usually of equal size and shield the cloacal complex dorsally, whereas abdominal skeletal muscle (MU) is found close to the ventro-lateral surfaces. The paired ureters (UR) and the oviducts (OD, Fig. 2) or vasa deferentia (VD, Fig. 3) in the reproductive tracts, are present ventro-lateral of the metanephri and open into the dorsal wall of the urodeum (UD) while the urinary bladder stalk (UBS) is found ventro-lateral of the cloacal complex.

The male cloacal complex of *C. cataphractus* (Fig. 3) is similar in structure to that of the female, with the largest dimorphism apparent in the urodeal region. Males do not have anterior urodeal limbs and the urogenital ducts empty conjointly into the urodeum through urogenital papillae.

1.1 Coprodeum (CO)

The lower intestinum of *C. cataphractus* leads posteriorly to the coprodeum. The coprodeum has more luminal folds than the lower intestinum and thick circularly arranged muscles distinguish the boundary between these two regions (Fig. 5), although the junction between the latter seems to be less defined in males than females. A thick circular muscle layer is present between the urodeum (UD) and the coprodeum as well as the coprodeum and urinary bladder stalk (UBS), thus clearly demarcating the boundaries between these structures (Fig. 6b).

1.2 Urodeum (UD)

Sexual dimorphism in the cloacal complex of *C. cataphractus* is mainly seen in the urodeal region (compare Figs. 2 & 3). With the female kidneys removed during dissection, the urodeum is noticed as a dorsal bulge of the cloacal complex, extending anteriorly (insert, region a, Fig. 2). Separation of the oviducts from the coprodeum reveals that the anterior urodeum of the female is divided into two cylindrical urodeal limbs (UD-L), surrounding the oviducts (OD) (Fig. 6a). These urodeal limbs merge (insert; region b, Fig. 2) to form a common urodeum (UD) as it extends posteriorly (Fig. 6b). This is a complex chamber with seemingly folded walls and it becomes flattened posteriorly. In contrast, the male urodeum is a barely-visible, dorsally displaced pouch (Fig. 3) containing no urodeal limbs and it has fairly prominent urogenital papillae (UP).

1.3 Copro-urodeum (CU)

As the cloacal complex of *C. cataphractus* extends posteriorly, about three-quarters through (inserts; region c, Figs. 2 & 3), the common wall between the posterior urodeum and coprodeum (Fig. 6b) becomes thinner in size (Fig. 7a) to form a passageway (see arrow, Fig. 7a) between these two regions, namely the copro-urodeum (Fig. 7b). The dorsal wall of the copro-urodeum is thicker than the ventral wall, exhibits taller folds and has more urodeal glands than the ventral wall.

1.4 Proctodeum (PR)

In both males and females, the posterior part of the metanephri becomes very small and fuses in the proctodeum region (Fig. 8b). The circularly arranged muscles in the wall of the copro-urodeum (CU) become thicker and highly folded, and the lumen reduces in size to form a sphincter-like muscular arrangement, indicating the start of the proctodeum (Fig. 8a). The proctodeal glands form a ring around this structure (Fig. 8b). The rounded proctodeal lumen becomes flattened more posteriorly (Fig. 9a), until it opens to the outside through a transverse cloacal opening/vent (Fig. 9b).

1.5 Urogenital ducts

The urogenital ducts of both sexes in *C. cataphractus* are paired structures that run posteriorly over the ventral surface of the kidneys, between the kidneys and the urodeum. In females, the paired urogenital ducts (oviducts and ureters) empty separately (see Fig. 2)

through the dorsal wall of the urodeum. In males, the urogenital ducts (vasa deferentia and ureters) unite before opening conjointly through the dorsal wall of the cloaca (see Fig. 3). The urogenital openings in the cloaca of male *C. cataphractus* are in more or less the same position as the opening of the ureters in the copro-urodeum in females.

1.5.1 Ureters (UR)

The ureters, leading from the kidneys, open independently through the urinary papillae into the dorsal wall of the copro-urodeum lumen (Figs. 2 & 3; 12). These ducts are dorso-lateral to the oviducts/vasa deferentia.

1.5.2 Oviducts (OD)

The paired oviducts (Fig. 6) of female *C. cataphractus* lie parallel to each other and are imbedded in a dorso-lateral bulge within each anterior urodeal-limb (UD-L), leading to the ovaries. The oviducts may be elongated at the point of emptying into the two urodeal limbs (see arrow, Fig. 6b).

1.5.3 Vasa deferentia (VD)

The paired vasa deferentia of males (Fig. 13b) join the ureters (insert, Fig. 14a) just before opening into the posterior part of the urodeal region (insert, Fig. 14b) through urogenital papillae. These tubes may be filled with spermatozoa (SZ) and secretion.

1.6 Urinary bladder stalk (UBS):

The urinary bladder stalk (Fig. 16) is a ventral evagination of the cloaca (ventro-lateral to the coprodeum) with ciliated cells (C) in the epithelial lining. The urinary bladder stalk connects the urinary bladder with the cloacal complex and becomes flattened just before opening into the coprodeum (Fig. 9b insert). The junction is usually anterior to the copro-urodeum, but in some individuals of *Cordylus cataphractus* it was found to be more posteriorly.

2. Micro-anatomy of the cloacal complex

The description of the anatomy of the cloacal complex is based on a series of anterior to posterior transverse sections (see inserts, Figs. 2 & 3).

2.1 Intestinum (IN)

The composition and orientation of the different layers of the intestinum are similar in male and female *C. cataphractus*. The intestinal epithelium (Fig. 4) is slightly folded and consists of columnar cells (with round to oval nuclei), with non-ciliated mucus-secreting goblet cells (see arrow, Fig. 4) scattered among them. The epithelial cells (Ep) are taller more posteriorly (towards the coprodeum) and appear as pseudostratified columnar epithelium. The layers underlying the intestinal epithelium consist of the lamina propria, a single layer of loose connective tissue extending into the luminal folds, followed by inner- and outer muscularis layers. A vascularized connective tissue layer separates the muscle layers. The inner muscularis (IM) consists of at least two thin,

smooth, circular muscle layers. The outer muscularis consists of bundles of longitudinal smooth muscle fibres. The serosa, a single thin layer of loose connective tissue, surrounding the intestinum, is not well defined.

2.2 Coprodeum (CO)

The coprodeum of both male and female *C. cataphractus* is lined with a pseudostratified columnar epithelium (Fig. 10b), with non-ciliated mucus-secreting cells (MS) scattered among columnar surface cells, but no goblet cells (as found in the intestinum) are present. The submucosa (lamina propria) (LP, Fig. 10b) is followed by the inner- and outer muscularis. The circularly arranged smooth muscle fibres of the inner muscularis (IM, Fig. 10a) extends into the luminal folds and is thicker than the inner muscularis of the lower intestinum. The inner muscularis is separated from the outer one by vascularized connective tissue, which seems to be thicker in the lateral areas. The outer muscularis (OM) is bundles of longitudinal smooth muscles. Some of these groups can be seen adjacent to the inner circular muscle layer in some areas and are more prominent in the lateral regions (Fig. 10a). The serosa is not easy to distinguish.

2.3 Urodeum (UD)

Each urodeal chamber (UD-L, Fig. 6a) of female *C. cataphractus* (insert, region a, Fig. 2) contains an expansive C-shaped lumen with smooth walls and few luminal folds. These walls are lined with a stratified, mostly cuboidal to columnar epithelium of several layers (2-5) in thickness. Conspicuous urodeal glands (UG) dominate the body of each

urodeal limb (Fig. 6a); the extent of development is related to season. The submucosa underlies the urodeal epithelium, and appears thicker or thinner depending on the extent of urodeal gland development. The single inner muscularis layer extends into the luminal folds and consists of circularly arranged smooth muscles in the ventral walls of urodeal limbs. The cells contain oval to round basal nuclei. The outer muscularis is formed by bundles of longitudinal smooth muscles. Again the serosa is not easily distinguished.

More posteriorly (insert, region b, Fig. 2), the urodeal limbs merge to form the common urodeum (UD, Fig. 6b). The ventral surfaces of the common urodeum is moderately to highly folded and generally has less urodeal glands while the dorsal surface exhibits lower folds and has more urodeal glands. The epithelium lining of the common urodeum is similar to the lining of the anterior limbs, the surface cells being non-ciliated and cuboidal to slightly columnar in shape. The inner- and outer layers of the muscularis, similar to the urodeal limbs, are found in the ventral walls of the common urodeum. Urodeal glands (UG) are less numerous as the cloacal walls become laterally compressed in the common urodeum.

The common wall between the urodeum and coprodeum (inserts, region c, Figs. 2 & 3), which primarily consists of circularly arranged smooth muscle fibers, becomes reduced, lacking urodeal glands ventrally (Fig. 7a) as the urodeum and coprodeum merge to form the copro-urodeum (CU, Fig. 7b). The luminal lining shows seasonal variation in height (apparently correlated with the reproductive cycle).

The male urodeum is lined with a pseudostratified columnar epithelium consisting of mucus-secreting cells. In contrast to the urodeum of the female, no urodeal glands

were found in the urodeal walls. The common wall between the coprodeum and urodeum is thicker in male *C. cataphractus* than in females.

2.4 Proctodeum (PR)

The proctodeum (inserts, region d, Figs. 2 & 3) exhibits a more folded epithelium than the coprodeum. The slightly folded mucosa of the anterior proctodeum consists of pseudostratified columnar epithelium, changing posteriorly into a stratified, cornified squamous epithelium at the vent (Fig. 11). The submucosa contains proctodeal glands that open through the posterior walls of the proctodeum. These glands form a ring around the proctodeum (Fig. 8b) and are more prominent in males than in females. The inner muscularis is a fairly thick layer of circularly arranged smooth muscles (Fig. 8b), extending into the luminal folds. It is usually thicker ventrally than dorsally and becomes reduced posteriorly (Fig. 9a) until it is no longer seen near the vent (Fig. 9b). The dorsal inner muscularis usually disappears from the proctodeum wall before the ventral inner muscularis does, as one scans from anterior to posterior. An outer muscularis is found in the ventral wall of the proctodeum and the serosa is not well defined. A blood plexus (BP) is located ventro-laterally to each side of the proctodeum (Fig. 9a). A hypoischium (HY; Fig. 7b) is situated ventro-medially in the proctodeum, with abdominal skeletal muscles (MU) extending from it.

2.5 Urogenital ducts

In contrast to the ureters, the terminal part of the oviducts (in females, Fig. 2) and vasa deferentia (in males; Fig. 3) possesses a relatively well-developed circularly arranged muscle layer.

2.5.1 Ureters (UR)

The three layers of the ureters (mucosa, muscularis and serosa) are well distinguished. The anterior mucosa layer (nearer to the kidneys) consists of simple, non-ciliated cuboidal to columnar epithelial cells (Fig. 15a), while more posteriorly (towards the cloacal junction), taller columnar mucus-secreting cells dominate (Fig. 15b). In the posterior urodeum (Fig. 7), the ureters turn more ventrally from the dorsal position as seen in Fig. 6 and empty into the copro-urodeum (CU) (arrow, Fig. 7b). The basic complexity and the underlying layers of the ureters are similar in male and female *C. cataphractus*.

2.5.2 Oviducts (OD)

The oviducts (female uteri) empty lateral to the ureters into the urodeum (arrow, Fig. 6b). The mucosa layer of the oviducts has numerous, tall folds extending into the lumen of each oviduct. The epithelium (Ep) consists of low, simple to pseudostratified, ciliated (C), columnar surface cells with oval to round basal nuclei (Fig. 13a). The epithelium of the oviduct becomes non-ciliated where it expands as the uterus. The papilla, through which the uterus enters the anterior urodeum, contains many tubules

lined with simple non-ciliated columnar to cuboidal epithelium cells. The submucosa extends into every luminal fold. The inner muscularis is a fairly thick layer of circularly arranged smooth muscle fibres, extending into luminal folds. The outer muscularis consists of bundles of longitudinal smooth muscles, and in some areas (embedded in submucosa) groups of muscle fibres were found external to the circularly arranged smooth muscles. A well-defined connective tissue layer is observed between the inner- and outer muscularis.

2.5.3 Vasa deferentia (VD)

The paired vasa deferentia in *C. cataphractus* males lie dorsal to the urodeum (Fig. 13b) and are lined with simple cuboidal epithelium. A relatively thin circularly arranged muscle layer, surrounding the vasa deferentia, becomes thicker posteriorly.

2.6 Urinary bladder stalk (UBS)

The urinary bladder stalk is situated directly beneath the large intestine (IN; Fig. 6a) and becomes flattened just before opening into the coprodeum (Fig. 6b insert). This junction is at approximately the same level where the coprodeum and the urodeum merge. The epithelium of the rounded (Fig. 16) anterior urinary bladder stalk (towards the urinary bladder) consists of ciliated, pseudostratified columnar epithelium. Some non-ciliated mucus-secreting cells occur among the ciliated epithelial cells. The lamina propria consists of a single layer of loose connective tissue. The inner muscularis (IM) extends into the luminal folds and is a fairly thick layer of circularly arranged smooth muscles.

The outer muscularis (OM) forms a prominent group of longitudinal muscles in a ventro-medial position and smaller groups in the dorso-lateral areas. Posteriorly, just before the coprodeum junction, these muscle groups become reduced.

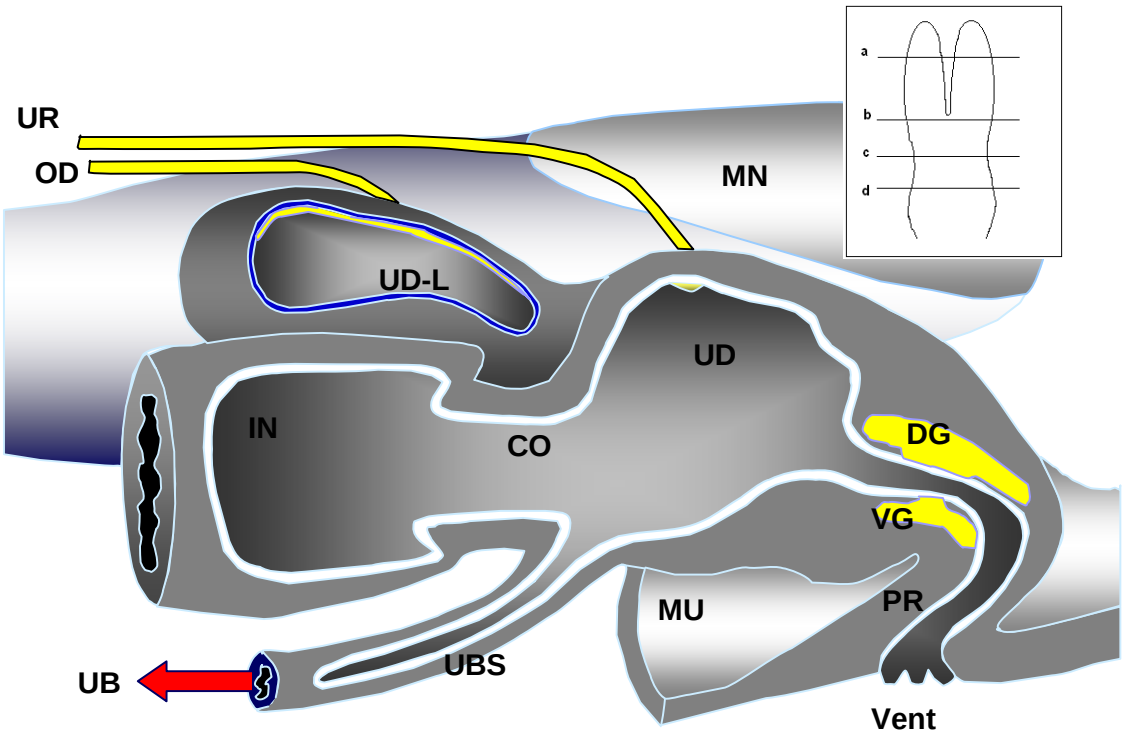

Figure 2. Schematic representation of a lateral view of the cloacal complex and associated structures of female *C. cataphractus.* Three regions are apparent, namely, the anterior coprodeum (CO), connecting the cloaca with the intestinum (IN), the intermediate urodeum (UD), divided anteriorly into two cylindrical urodeal limbs (UD-L), and the posterior proctodeum (PR), leading to the outside through the vent. The urogenital ducts open separately into the cloacal complex; the oviducts (OD) into the urodeal limbs and the ureters (UR) into the urodeal region. A simplified dorsal view diagram is inserted. Note the positions of the sections for later histological description of the complex. UG = Urodeal glands; DG = dorsal glands; VG = ventral glands; UBS = urinary bladder stalk; MU = abdominal muscularis layer; MN = metanephros.

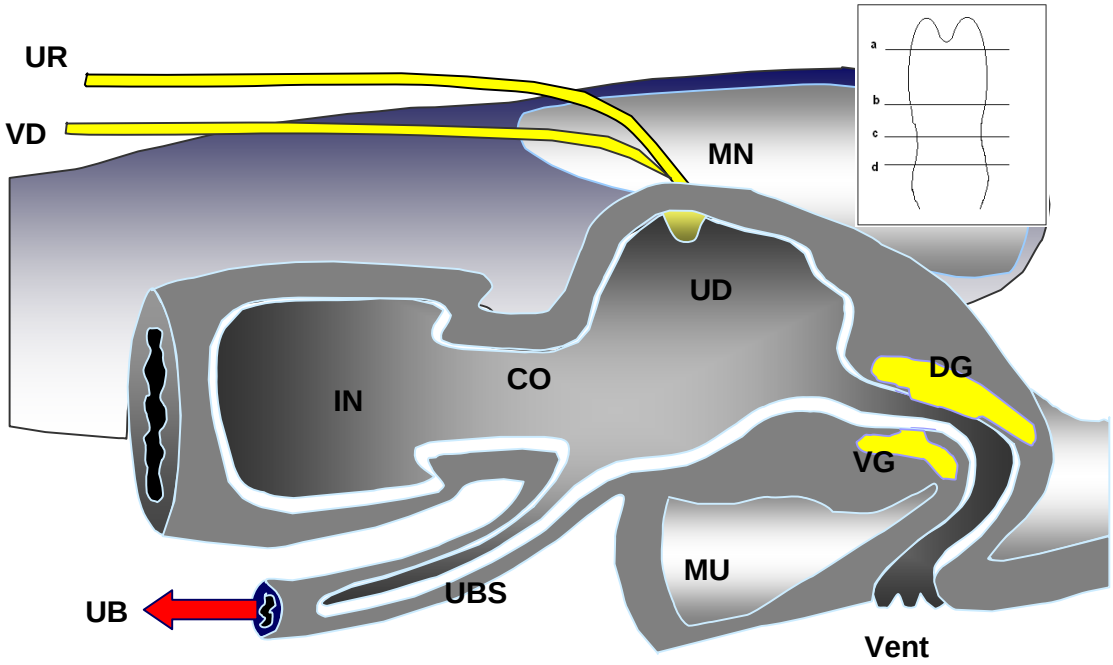

Figure 3. Schematic representation of a lateral view of the cloacal complex and associated structures of male *C. cataphractus.* Although the structure is similar to that of the female, the most striking differences are found in the urodeal (UD) region. The male cloaca has no urodeal limbs and the urogenital ducts, ureters (UR) and vasa differentia (VD), open conjointly into the urodeal region usually through distinctive urogenital papillae (UP). The dorsal (DG) and ventral (VG) proctodeal glands in the proctodeal region are larger than those of the female and no urodeal glands are found in males. A simplified dorsal view diagram is inserted. MU = abdominal muscularis layer; UBS = urinary bladder stalk; MN = metanephros.

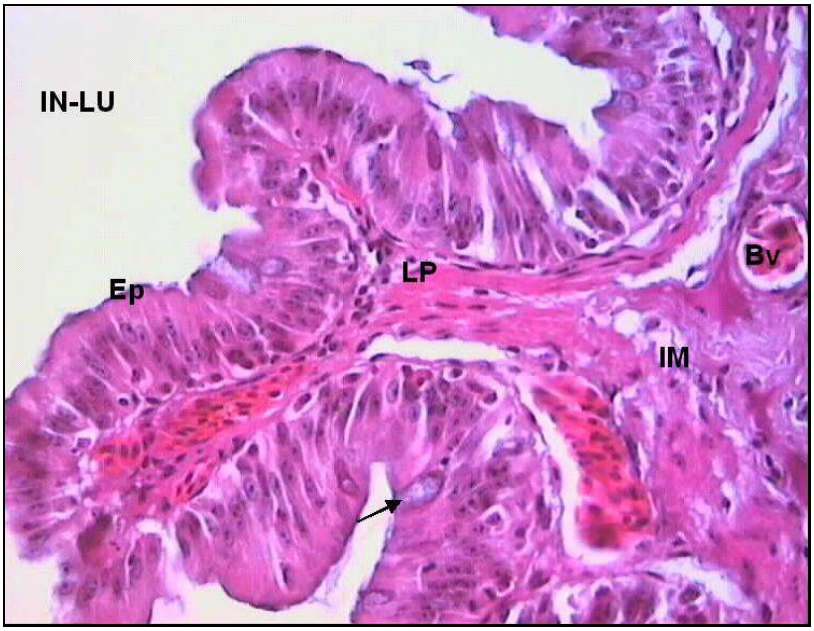

Figure 4. The posterior intestinum of *Cordylus cataphractus,* stained with H & E. Note the goblet cell (arrow) in the epithelium (400x). Bv = bloodvessel; LP = lamina propia; Ep = epithelium; IM = inner muscularis; IN-LU = intestinum lumen.

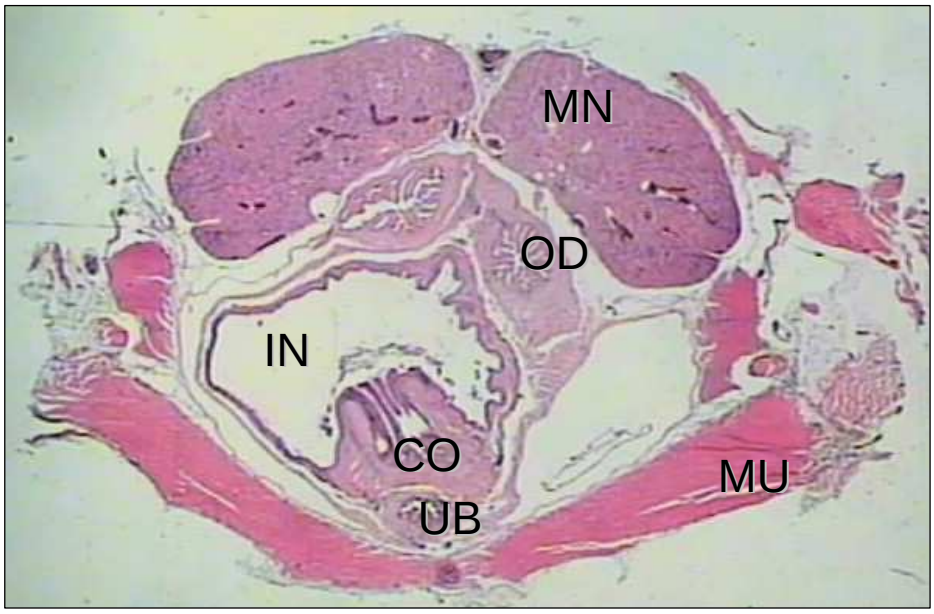

Figure 5. The cloacal complex of female *Cordylus cataphractus*, showing the junction between the intestinum (IN) and the coprodeum (CO) (60x). MN = metanephros; MU = abdominal muscle layer; OD = oviduct; UBS = urinary bladder stalk.

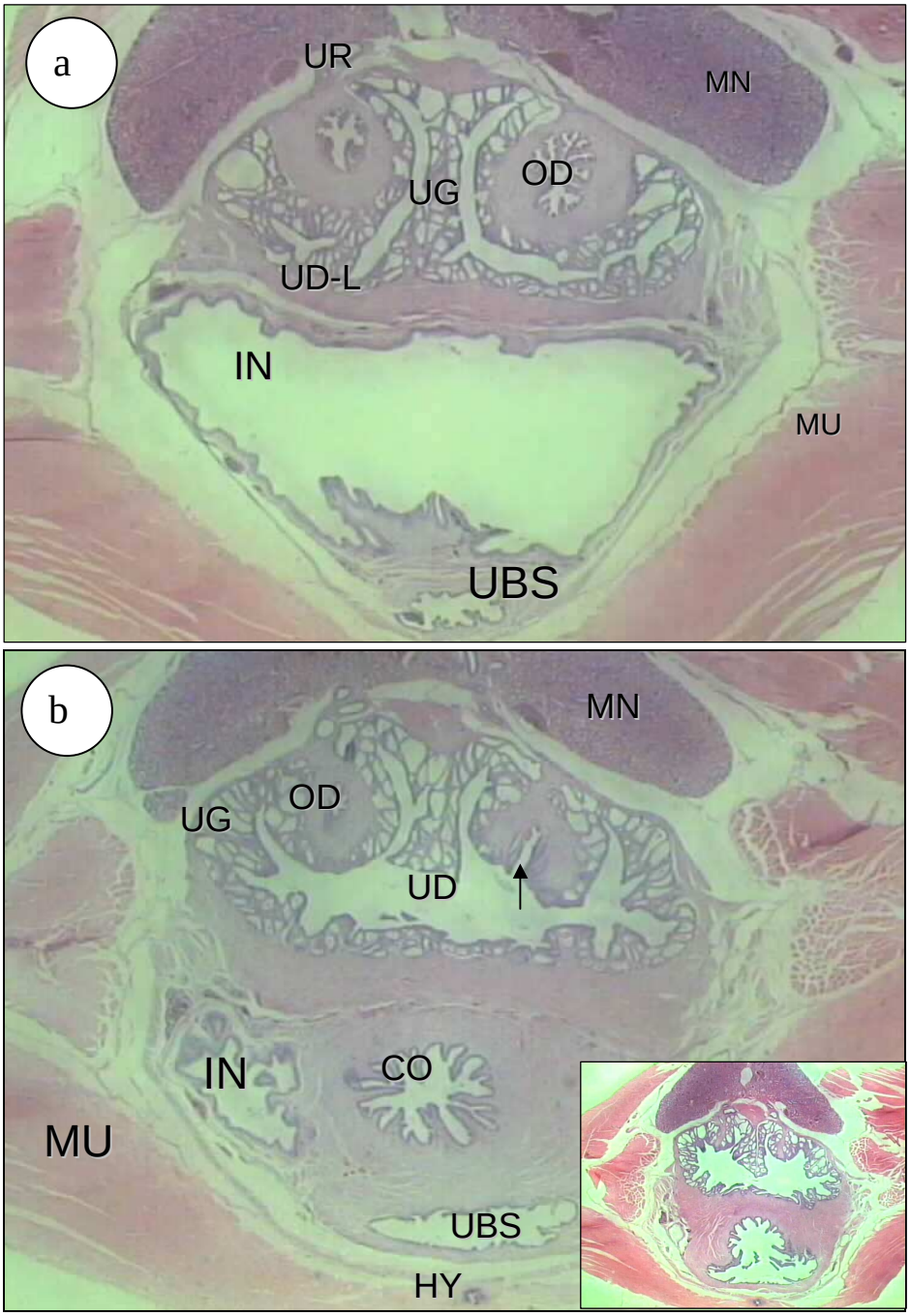

Figure 6. The anterior part of the cloacal complex of a late-vitellogenic female *Cordylus cataphractus*. a) The paired urodeal-limbs (UD-L) merge to form the b) common urodeum (UD) as they extend posteriorly. The oviducts (OD) open into the urodeal lumen (see arrow). The urinary bladder stalk becomes flattened just before opening into (b, insert) the coprodeum (all at 120x). CO = coprodeum; HY = hypischium; IN = intestinum; MN = metanephros; MU = abdominal muscles; UG = urodeal glands.

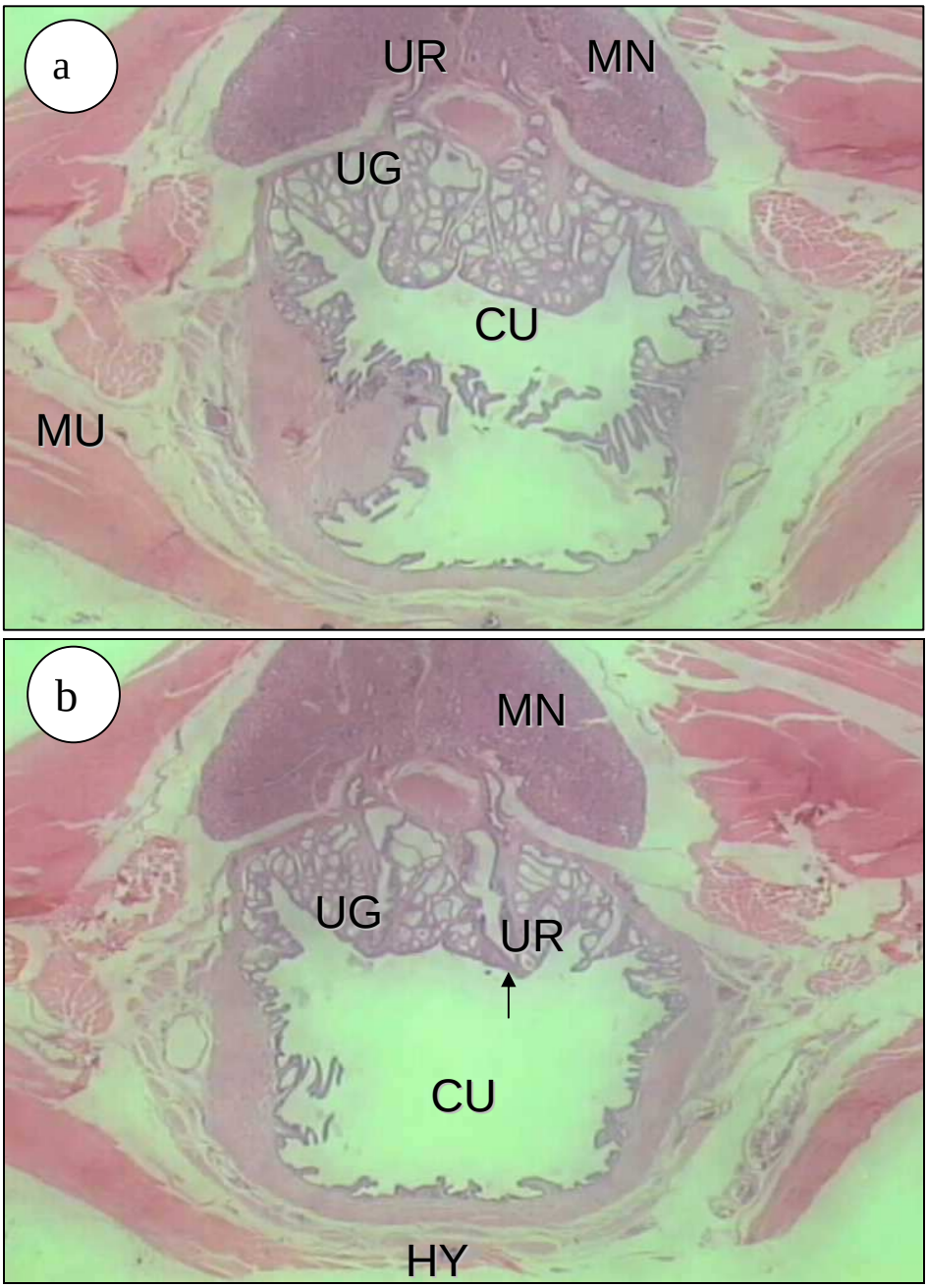

Figure 7. The middle part of the cloacal complex of a late-vitellogenic *Cordylus cataphractus* female. a) The common wall between the urodeum and coprodeum becomes thinner and forms b) a copro-urodeum (CU) as the cloacal complex extends posteriorly. Note the position of the ureters (UR) opening into the copro-urodeum (arrow) (both 120x). HY = hypischium; MN = metanephros; MU = abdominal muscles; UG = urodeal glands.

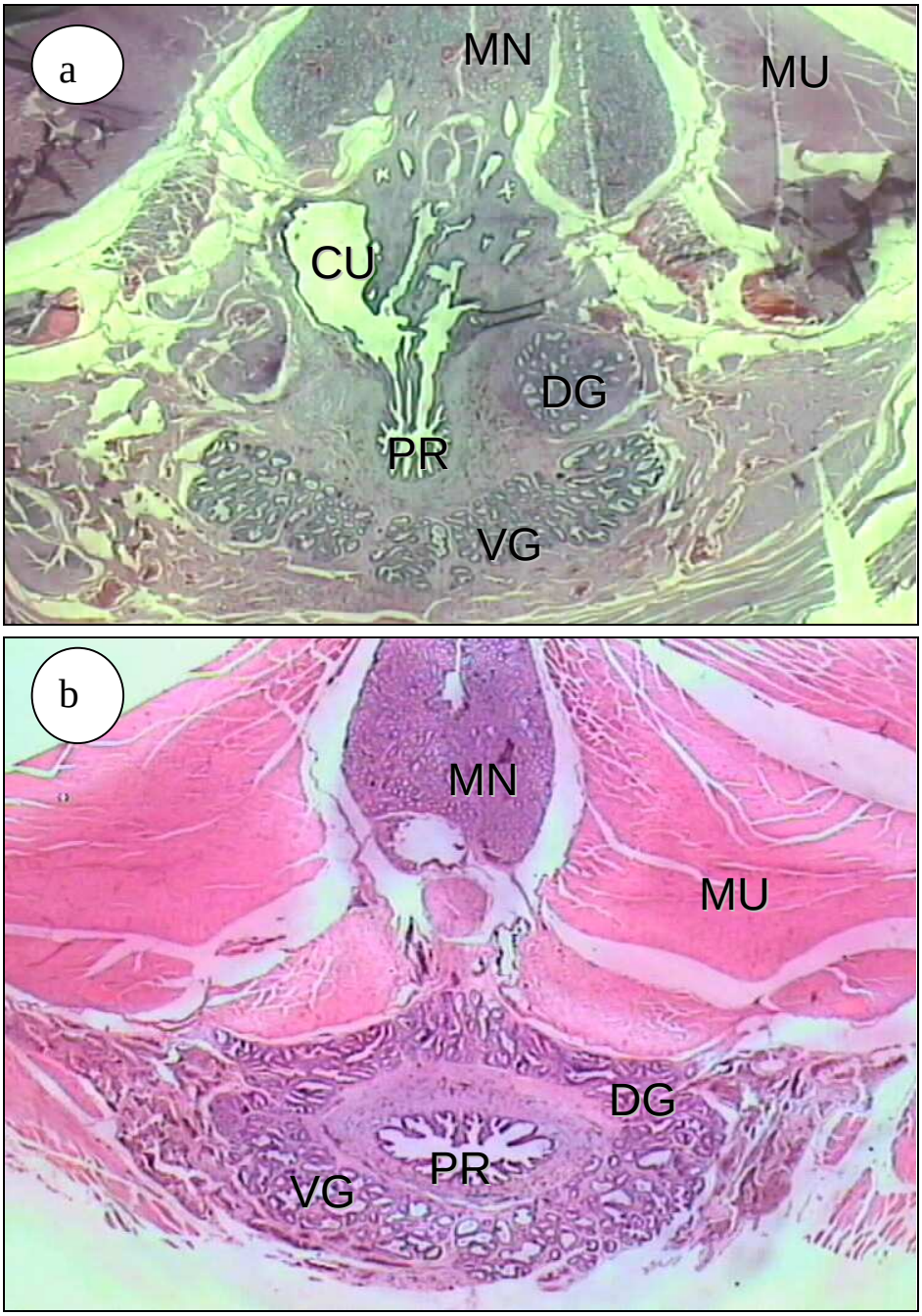

Figure 8. The posterior part of the cloacal complex of a late-vitellogenic female *Cordylus cataphractus.* a) The circular muscle layer around the copro-urodeum (CU) becomes thicker and highly folded, indicating the start of the proctodeum (PR) (120x). b) More posteriorly, the proctodeal glands (DG and VG) form a ring around the proctodeum. Note fusion of the kidneys (MN) and the increase in the abdominal skeletal muscles (MU) (120x). Both photomicrographs were stained with H & E, different batches.

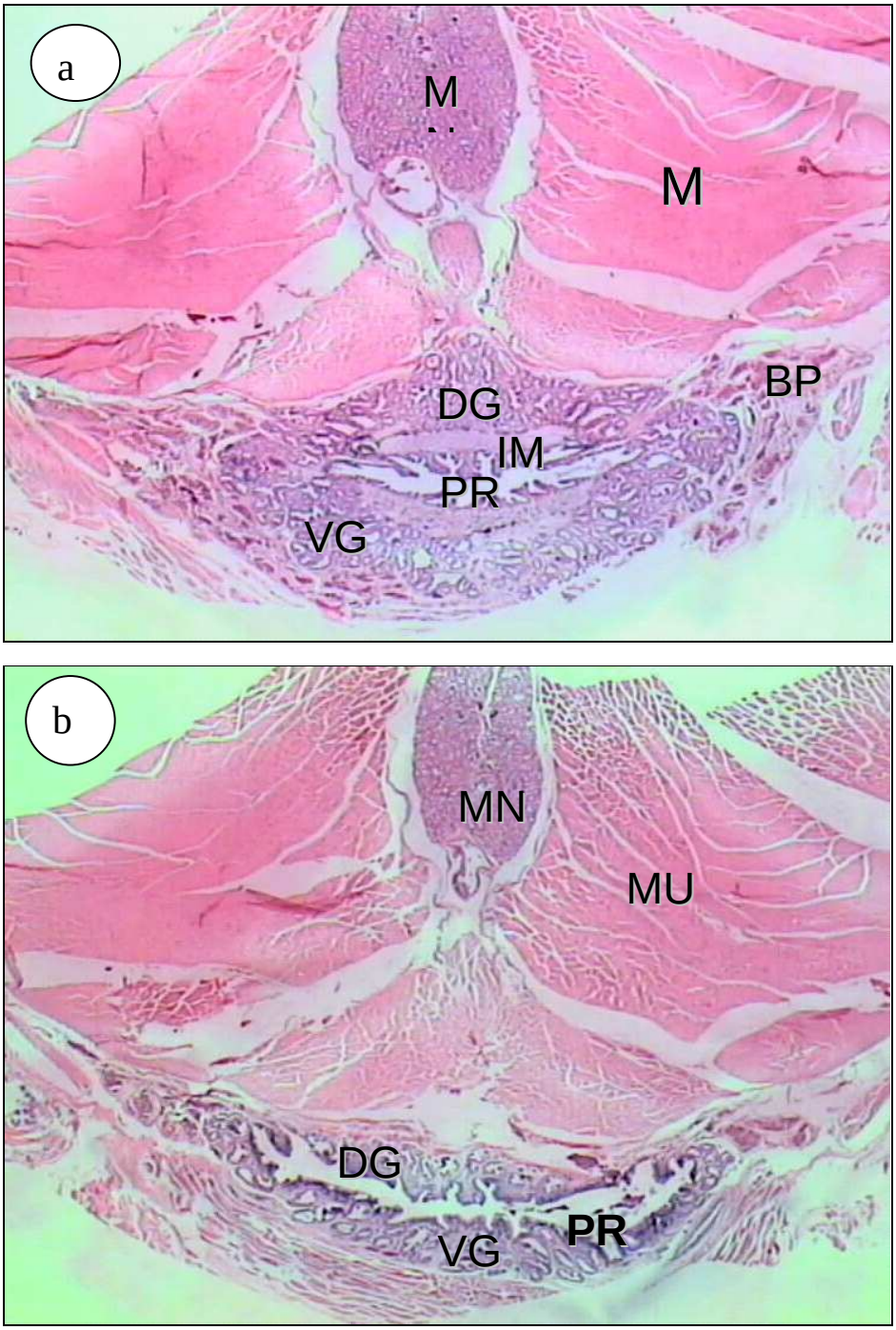

Figure 9. The cloacal complex of a late-vitellogenic female *Cordylus cataphractus* near the vent. The thick muscle layer (IM) around the proctodeum (PR) becomes reduced towards the vent as the proctodeum flattens (120x). BP=blood plexus; DG= dorsal glands; VG= ventral glands; MN = metanephri; MU = abdominal muscles.

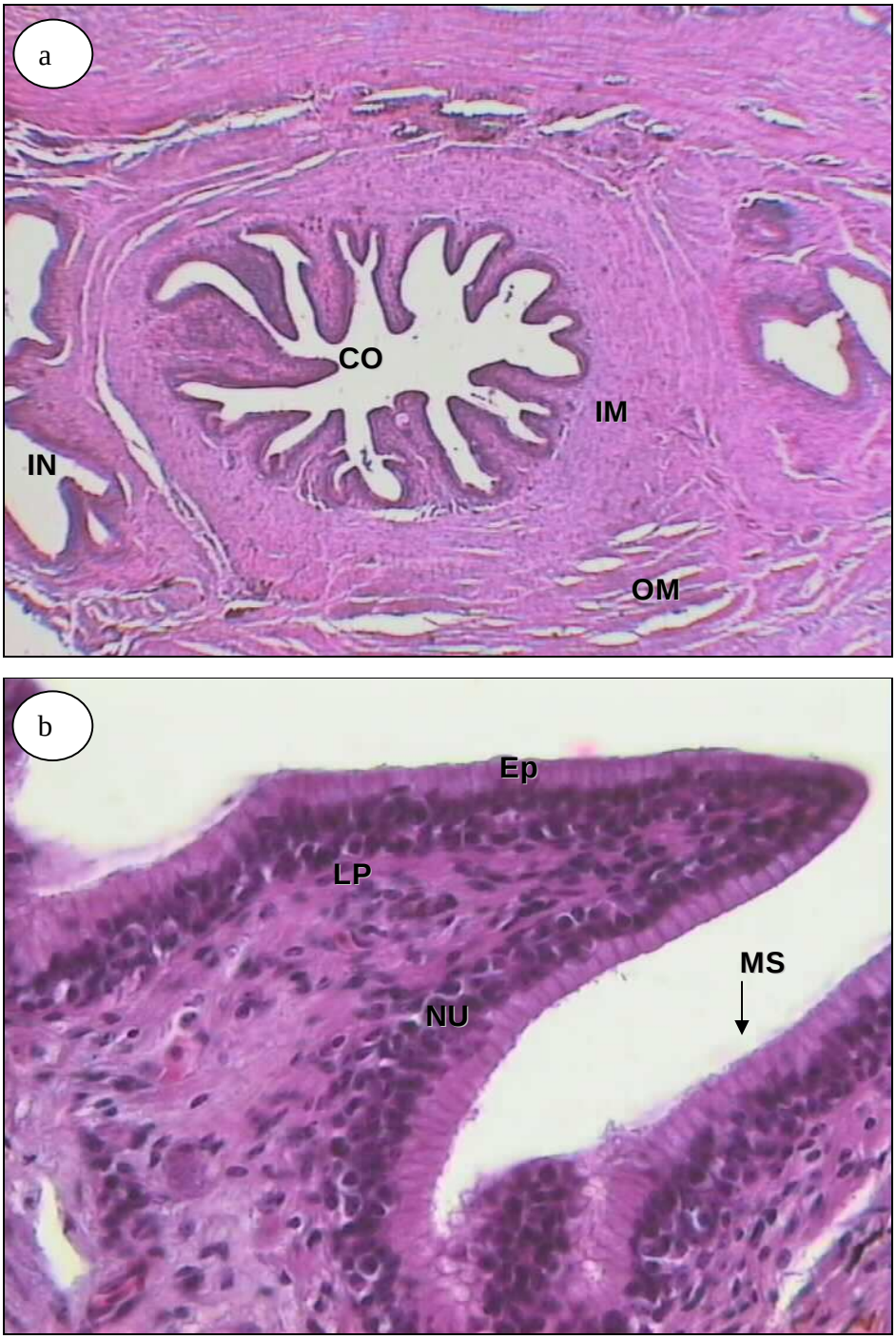

Figure 10. The coprodeum (CO) of a *Cordylus cataphractus* female, showing the a) luminal folds (40x) and b) the underlying layers of the epithelium. Note the mucus-secreting cells (MS) in the epithelium layer (Ep) (400x). IM = inner muscularis; IN = lower-intestinum; LP = lamina propria; NU = nucleus; OM = outer muscularis.

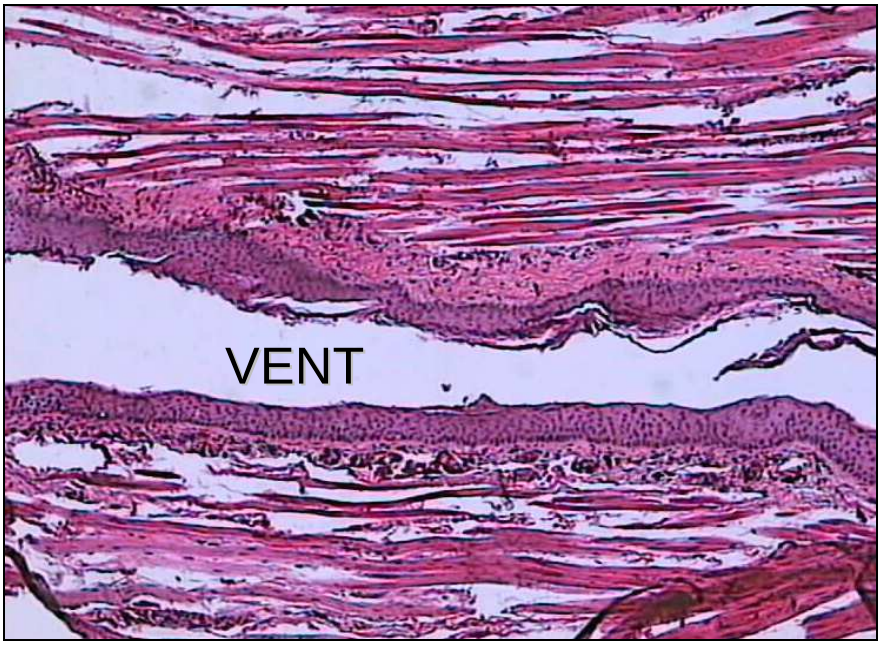

Figure 11. The cloacal opening of a male *Cordylus cataphractus.* At the vent the proctodeal epithelium becomes stratified and squamous as it leads to the outside (100x).

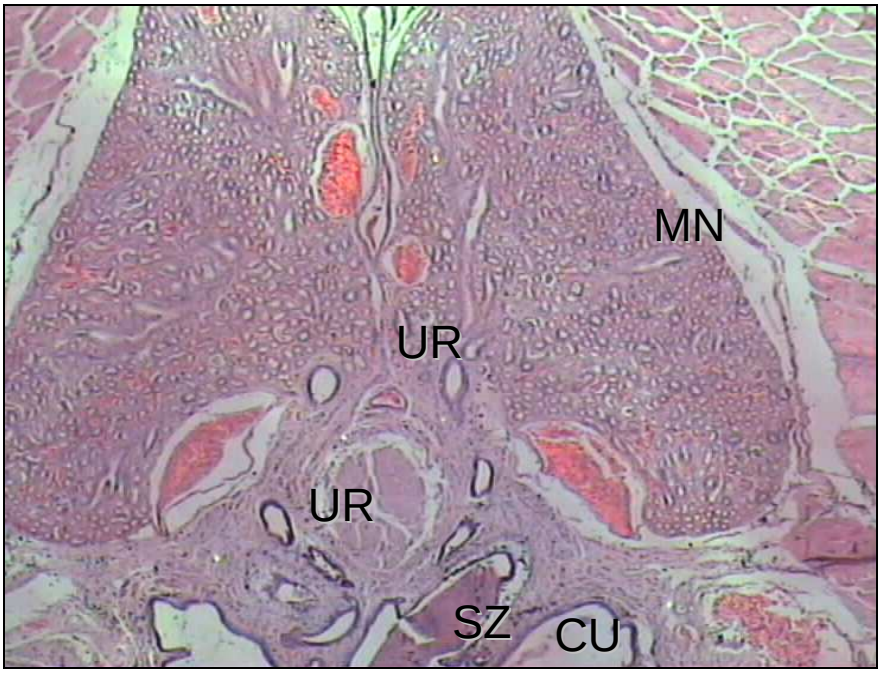

Figure 12. The parallel ureters (UR) are seen posteriorly at the ventral surface of the kidneys (MN) and independently enter the dorsal wall of the cloaca of a male *Cordylus cataphractus* at the copro-urodeal region (CU) (250x). SZ = spermatozoa.

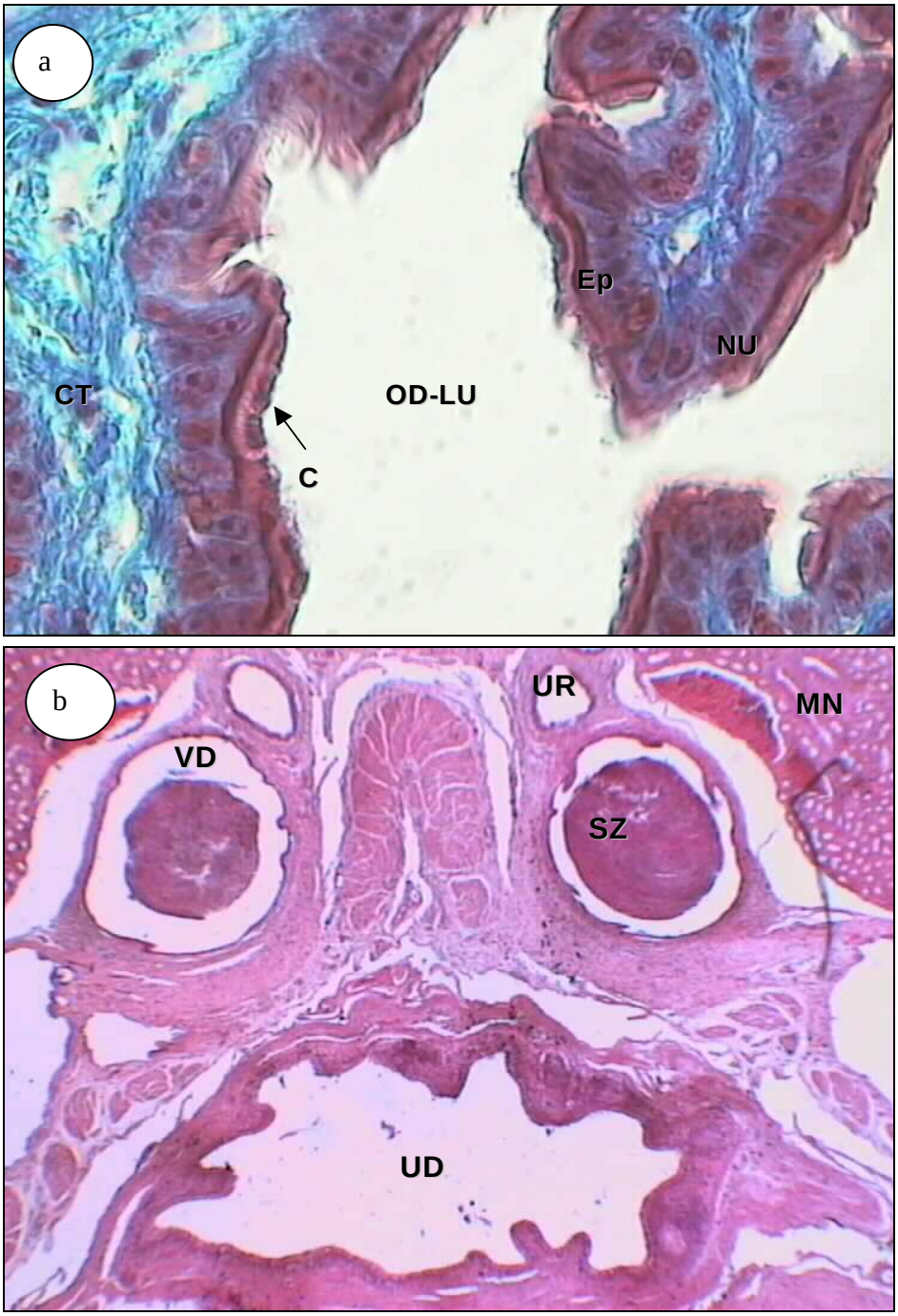

Figure 13. a) Female oviducts, stained with Masson's Trichrome (1000x) and b) male vasa deferentia, stained with H & E (40x) of *Cordylus cataphractus.* Note the ciliated (C) columnar epithelial lining of the oviduct and the spermatozoa (SZ) in the vasa deferentia (VD). CT = connective tissue; OD-LU = oviduct lumen; MN = metanephros; UD = urodeum; UR = ureter.

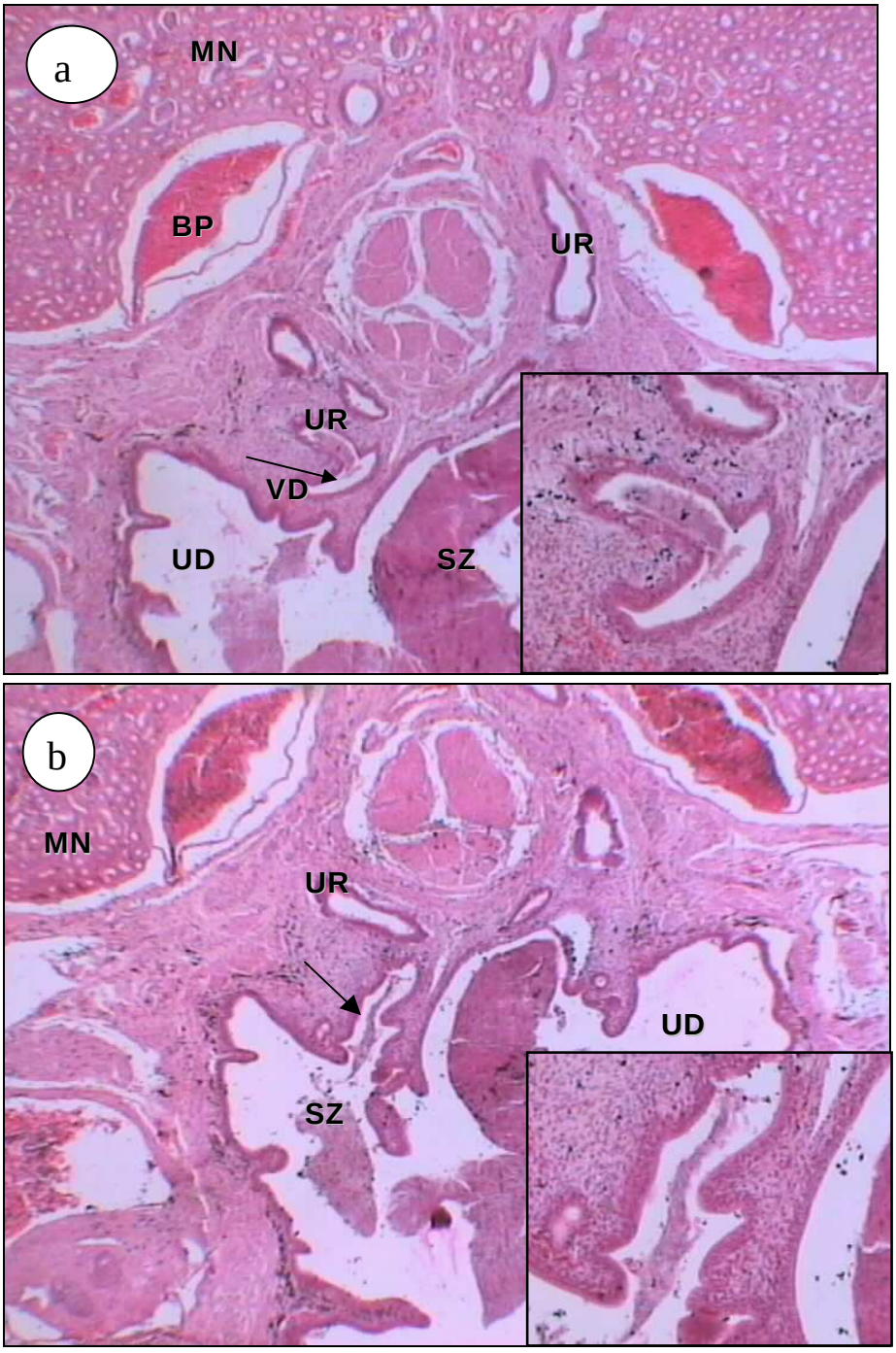

Figure 14. Urodeum of a male *C. cataphractus* a) at the point where the ureter (UR) and vasa deferentia (VD) coalesce (see arrow) (40x), and b) where they conjointly empty into the urodeal lumen (UD) (see arrow) (40x). The inserts show the same structures at higher magnification (100x). Note the high volume of spermatozoa (SZ) in the urodeum. BP = blood plexus; MN = metanephros.

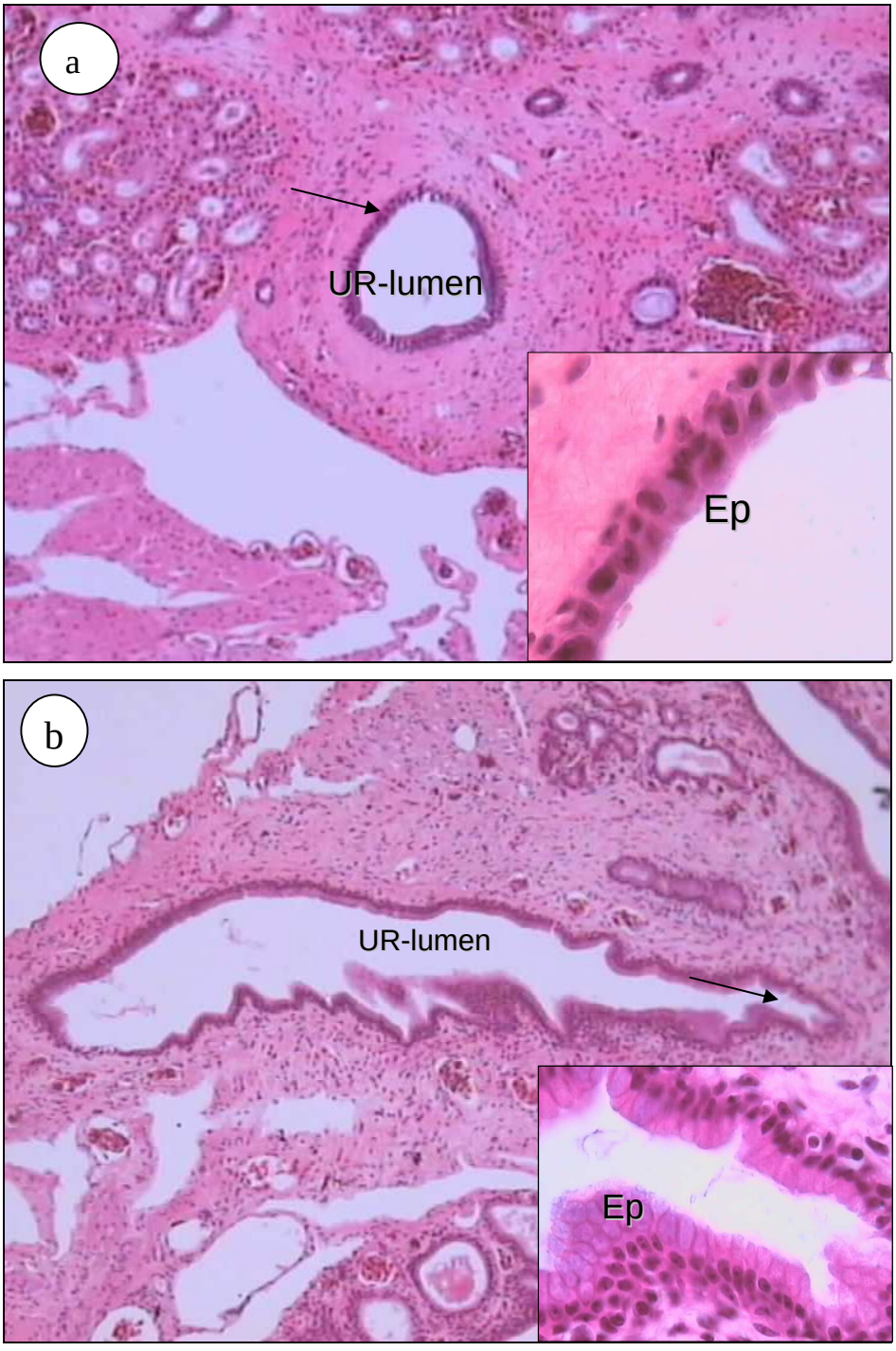

Figure 15. a) Anterior and b) posterior part of ureter (UR) of *Cordylus cataphractus* (100x). Note the difference in epithelium height (Ep) between the rounded anterior (insert, Fig. 15a) and posterior (insert, Fig. 15b) ureters (both inserts at 1000x). Arrows show point of magnification.

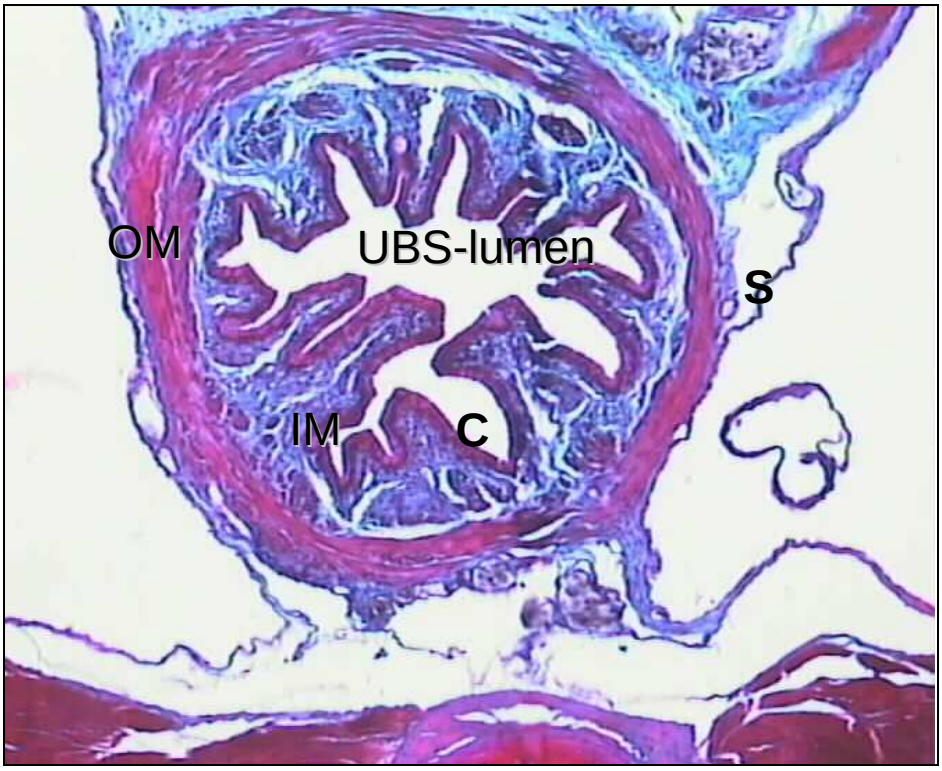

Figure 16. The anterior part of urinary bladder stalk (UBS) in a *Cordylus cataphractus* female, stained with Masson's Trichrome (100x). C = cilia; IM = inner muscularis; OM = outer muscularis; S = serosa.

- DISCUSSION –

1. Gross anatomy of the cloacal glands

The three-chambered design of the cloacae of *Cordylus cataphractus* males and females are almost identical to that of congenerics studied, namely *C. cordylus* and *C. melanotus* (Mackay, 1993). Boundaries between the lower intestinum and coprodeum, as well as between the urodeum and proctodeum are relatively distinct in *Cordylus*, but the coprodeum to urodeum transition is not well delimited in any of these species. Mackay (1993) mentioned that the intestinum to coprodeum transition appears a little more obscure in males of *C. cordylus* and *C. melanotus* than in females, which is also the case in *C. cataphractus*.

Differences in cloacal topography between the mentioned *Cordylus* species and *Gerrhosaurus nigrolineatus* (Cooper & Trauth, 1992) are also relatively minor. This is not surprising as the mentioned taxa belong to the families Cordylidae and Gerrhosauridae, respectively, and these are considered sister families (Lang 1991). Although topographical differences between representatives of other lizard families and *C. cataphractus* are evident, these have little meaning in the absence of a comprehensive phylogenetic analysis. The urogenital ducts in the three species of *Cordylus* empty at virtually the same positions in the urodeum. In females, the oviducts and ureters open separately - oviducts more anteriorly and ureters more posteriorly in the dorsal wall of the copro-urodeum. *Gerrhosaurus nigrolineatus* (Cooper & Trauth, 1992) females

apparently deviate from this pattern - oviducts empty anteriorly into the urodeal limbs, but ureters in the same position as in *Cordylus*. One should treat this difference with caution, though, as Mackay (1993) only studied two females and intraspecific variation in position of entry of urinogenital ducts may exist. The bladder stalk leaves the copro-urodeum ventrally in the same position in all cordylids and the gerrhosaurid studied.

2. Micro-anatomy of the cloacal complex

Histological appearance of the walls of the cloacal chambers of *C. cataphractus*, and that of urinogenital ducts and the intestinum opening into the cloacal complex, conform to that described for *C. cordylus* and *C. melanotus* (Mackay, 1993). Cooper & Trauth (1992) gave more superficial histological descriptions for *Gerrhosaurus nigrolineatus*, but it is clear that this species have similar cytological appearance, except for some differences in the male urodeum.

The urodeum of *C. cataphractus* share the following similarities with *G. nigrolineatus* (Cooper & Trauth, 1992), *C. cordylus* and *C. melanotus* (Mackay, 1993): conspicuous urodeal glands occur in the walls of the female urodeal limbs; there are less glands in the common urodeum of females than in the urodeal limbs; and no urodeal glands are present in males. *G. nigrolineatus* males, however, differ from *Cordylus* in having gland-like invaginations, similar to the urodeal glands in its females (Cooper & Trauth, 1992). Glandular structures have never been described in any other lizard male in this region. Further, the urogenital papillae of *G. nigrolineatus* males are very prominent and so-called posterior urodeal cavities present. Papillae are small in *Cordylus* males and there

are no signs of posterior urodeal cavities. These urodeal differences may reflect phylogenetic distance, the difference in life-style (gerrhosaurids being solitary-living and *C. cataphractus* group-living), or the difference in reproductive mode (gerrhosaurids being oviparous and cordylids viviparous); the latter hypothesis can only be tested if cloacal anatomy of an oviparous member of Cordylidae (i.e., *Platysaurus*) is studied.

3. Cloacal glands

The glands of the proctodeum also show differences among the gerrhosaurids and cordylids studied. *Gerrhosaurus nigrolineatus* females have sparse proctodeal glands, but those of males are large (Cooper & Trauth, 1992). In *C. cataphractus*, proctodeal glands are large in both sexes, albeit less prominent in females. *C. cordylus* and *C. melanotus* share this condition (Mackay, 1993). Another aspect that varies among these species is the relative positions of the dorsal and ventral gland masses to each other. Cooper & Trauth (1992) found that the dorsal gland clusters lie more anteriorly than the ventral ones, and that the ventral glands extend further backwards than the dorsal glands. Mackay (1993) found in both sexes of *C. cordylus*, and the males of *C. melanotus*, that dorsal glands extend further backwards than do ventral glands. *C. melanotus* females, however, exhibit the opposite condition. These apparent differences must be put into perspective as *C. cataphractus* males and females show much variation. About half of the individuals studied showed dorsal glands extending further posteriorly, and the other individuals the reverse condition. As few individuals were examined by Cooper & Trauth (1992) and Mackay (1993), and little is known about intraspecific variation regarding relative

proctodeal gland positions in these species, the recorded differences must be treated with caution.

In conclusion, observed differences among cordylid and gerrhosaurid species studied by Cooper & Trauth (1992), Mackay (1993), and the present study allow the following generalizations: There are almost no structural and histological differences among members of *Cordylus*. As such, differences between sexes are consistent among the *Cordylus* species and the sexual dimorphism observed, can be considered conservative. *Cordylus cataphractus*, although having a group-living lifestyle, does not differ in any significant way from solitary living congenerics when it comes to cloacal topography and histology, including the terminal urinogenital ducts and glands asociated with the cloaca. Finally, it is at present not possible to relate the few structural and histological differences noted between *Gerrhosaurus nigrolineatus* on the one hand and *Cordylus* on the other, to parity mode, life-style differences, or merely on phylogenetic distance.

CHAPTER 3: STRUCTURE AND SECRETORY ACTIVITY OF URODEAL GLANDS IN *CORDYLUS CATAPHRACTUS*

- INTRODUCTION -

Two types of cloacal glands are found in the cloacal complex of lizards, namely the urodeal and proctodeal glands (Whiting, 1969; Trauth, *et al*., 1987; Cooper & Trauth, 1992; Mackay, 1993). As their names suggest, these exocrine glands are found in the walls of the two more posterior compartments of the three-chambered lizard cloaca, the urodeum and proctodeum, respectively (see Kardong (2000) for a brief description of the topology of the lizard cloaca). The most anterior compartment, the coprodeum, houses no glands.

Proctodeal glands are present in both sexes, but urodeal ones in females only (Hardy & Cole, 1981; Trauth *et al*., 1987; Cooper & Trauth, 1992; Mackay, 1993). Urodeal glands are generally absent in juveniles and sub-adults (Whiting, 1969; Mackay, 1993), or inactive when occurring in sub-adults (e.g., in the scincid *Eumeces laticeps* (Trauth *et al*., 1987)). This suggests that secretions are only produced after sexual maturity is reached (Cooper *et al.*, 1986). The female urodeum is a complex compartment with two cylindrical anterior limbs, not present in males, (Gabe & Saint Girons, 1965) and urodeal glands are especially conspicuous in these urodeal limbs. Two

types of urodeal gland organizations are known in lizards: a) branched tubular, as found in a gerrhosaurid (Cooper & Trauth, 1992) and cordylids (Mackay, 1993), and b) tubulo-alveolar as is evident in scincids (Trauth *et al.*, 1987) and teiids (Hardy & Cole, 1981). Regamey (1935) and Gabe & Saint Girons (1965) found that urodeal glands of lacertids are holocrine, while Hardy & Cole (1981) described those in teiid species as apocrine, holocrine or both. The tubules of urodeal glands generally contain simple non-ciliated, columnar epithelia with mucus secreting cells, and the nuclei of the glands are round to oval and exhibit basal positions (Trauth *et al.*, 1987; Cooper & Trauth, 1992; Mackay, 1993).

Although cloacal anatomy has been described for numerous lizards since the late 1800's, very few data exist on secretory activity and the functional significance of urodeal glands. Some authors proposed either a lubrication function (Regamey, 1935; Mackay, 1993), or that of pheromonal communication (Cooper *et al.*, 1986; Trauth *et al.*, 1987; Cooper & Trauth, 1992). Significantly, urodeal glands are known to be cyclic in secretory activity. Regamy (1935) was first to describe seasonal changes in the urodeal epithelium of female *Lacerta agilis*. Gabe & Saint Girons (1965) described some seasonal variation in cloacal anatomy, stating that urodeal epithelial lining became modified during the course of the reproductive cycle. Recently, Cooper *et al.* (1986) and Trauth *et al.* (1987) observed that the epithelial lining of urodeal glands in females of the scincid *Eumeces laticeps* underwent dramatic seasonal enlargement, becoming highly secretory during the reproductive season. Cooper & Trauth (1992) made similar observations for the cordyliform lizard *Gerrhosaurus nigrolineatus*, but Mackay (1993)

reported that urodeal glands were completely regressed in gravid *Cordylus cordylus* females. These females apparently have enlarged urodeal glands during pre-vitellogenesis. The only three cordyliform species (i.e., *C. cordylus, C. melanotus* and *G. nigrolineatus*) studied to date show different patterns in urodeal gland activity. Also, different functions have been proposed for its secretions in these species. More cordyliform species need to be investigated before the significance of seasonal variation in urodeal gland activity can be understood.

The literature report basically two approaches for determining whether changes in cloacal glands of lizards are seasonal or not, the nature of their secretions, and finally, their possible functions. Comparison of histological appearance of glands in females of different reproductive status has been the favoured method. Often, only two or three stages were compared, and sample sizes were normally very small. The second approach has been the use of histochemistry to determine the nature of secretion, and then to deduce a possible function from it. Statements on differences in extent of glands and epithelial heights at different times of the year have been qualitative, not a single paper reporting the use of proper morphometric procedures. There are also no studies on experimental manipulations of secretions in these glands in order to determine its possible role.

The Armadillo Lizard, *Cordylus cataphractus*, is a cordylid species suitable for studying urodeal gland histology and function for two reasons. Firstly, detailed histological observations exist for two congenerics (i.e., those studied by Mackay, 1993). Second, much is known about the life history of this species (Peers, 1930; Branch, 1988;

Mouton *et al.*, 1999; Visagie, 2001; Flemming & Mouton, 2002). In the present study, I also follow a comparative approach, comparing histological appearance at different reproductive stages in females of this lizard, using large sample sizes. Through histochemistry, I tried to determine the nature of urodeal gland secretions. By comparing tubular diameters and epithelial heights in urodeal glands among females at different reproductive stages, I was able to determine whether or not urodeal glands are cyclic in secretory activity.

The above aims enabled me to comment on a possible role in lubrication and/or pheromonal communication of these glands. I made the following predictions in this regard: If the function of urodeal glands is for lubrication, one would expect that large volumes of mucoid secretion will be formed in the glandular tubules; the glands will be absent or inactive in one sex if it was related to a sex-specific aspect of reproduction; secretory activity will be seasonal if the function relates to a seasonally occurring event, or the secretory activity will be continuous if the function relates to events occurring frequently. If the function is pheromonal communication, one would expect that the secretion will include lipoidal components; that the glands will be absent or inactive in one sex if they were conveying sex-related information; that the secretory activity will be seasonal if the glands convey information on sexual or social status or that secretory activity will be continuous if the function relates to events occurring frequently.

- MATERIALS AND METHODS -

1. Materials

Specimens were collected in the Lamberts Bay - Graafwater district and accessioned into the Ellerman Collection of Stellenbosch University (see Chapter 2 for details). The cloacal complex of a single adult male (USEC/H-3070) was excised and compared to that of females. At least four females representing each of the six different reproductive stages (as defined by Flemming & Mouton, 2002), were excised (see Table 2). Appendix 2 reports additional detail on specimens used, collection dates and locality data.

2. Histological preparation of tissue samples

Excised tissues were subjected to standard histological techniques (see Chapter 2 for details), except for the cloaca of a single female which was subjected to freeze-microtomy. Cloacae were transversely sectioned at a thickness of 6 to 11 μm, and a complete serial sequence of the urodeum of at least one specimen per reproductive stage was obtained. Other cloacae were routinely sectioned in the urodeal region. The following staining procedures were followed: Erlich's Haematoxylin and Eosin (H & E), Mallory's Triple Stain, and Periodic Acid Schiff (PAS) were alternated on groups of slides. The cloaca processed for cryostat sectioning was of a mid-gravid female, and had

been fixed in formalin. Routine sections of this cloaca were cut at 8 µm and were subsequently stained with Sudan Black B for presence of lipids and mounted with glycerin jelly. Photographs were taken using digital cameras and Leica Quips Image Analysing Software, mounted to stereo- and compound microscopes.

3. Statistical analyses

At least 10 measurements of diameters and epithelial heights per cross section of urodeal gland tubules were obtained (to the nearest 0.001 mm) in four regions of the gland (anterior urodeal limbs, common urodeum, posterior urodeum and at the coprodeum-proctodeum junction), using a compound microscope and Leica Quips Image Analysing Software. Nested ANOVA (with the region and individuals as independent factors) was used to test for differences in diameters and epithelial heights among reproductive stages, and $P < 0.05$ was considered significant.

Table 2. Number of female *Cordylus cataphractus* individuals at different reproductive stages used for histological evaluation of cloacal gland presence and activity.

Reproduction status	Collection month	Sample size (n)
Pre-vitellogenic	Jan to Apr	4
Early vitellogenic	May to Jul	4
Late vitellogenic	Apr to Oct	5
Early gravid	Oct to Jan	4
Mid-gravid	Jan to Mar	5
Late gravid	Mar to Apr	5

- RESULTS -

1. Gross anatomy of the female urodeum and its glands

The anatomy of the urodeum in *Cordylus cataphractus* females is described in detail in Chapter 2. The female urodeum is seen anteriorly as two cylindrical urodeal limbs (UD-L; Fig 17a), extending from a dorso-antero direction from a common urodeum chamber (UD; Fig. 17b). The latter joins the coprodeum more posteriorly to form a "copro-urodeum" (CU; Fig. 17c) – this is because the boundary between the coprodeum and urodeum is not distinct. The urodeal lumen is obvious in all of these compartments, but the shape and extent differ (Fig. 17). The submucosa of the urodeum has conspicuous longitudinal and circular muscle layers, which vary in thickness among the urodeal compartments. The walls, specifically the submucosa of the urodeal limbs and common urodeum chamber, primarily consist of glandular tissue. These compound tubulo-alveolar glands are called urodeal glands and the size and activity of these glands appear to vary seasonally. The submucosa of the copro-urodeum houses much less glandular mass, and the muscularis thickens towards the proctodeum to form a sphincter between the compartments (Fig. 18). At the beginning of the posterior proctodeum, these urodeal glands are only present in the dorsal folds (arrow, Fig. 18).

2. Micro-anatomy of the female urodeum and position of its glands

The anterior region of the paired urodeal limbs (Fig. 17a) has a C-shaped lumen, enclosing each oviduct. The urodeal limbs have a smooth mucosa and small luminal folds while the paired oviducts have highly folded mucosas. Urodeal glands dominate the submucosa of each limb at the height of reproductive activity. In the common urodeal region (Fig. 17b), the urodeal lumen is dorso-ventrally compressed and the ventral mucosa appears more folded. The submucosa on the ventral surface generally contains less urodeal glands than dorsally. The mucosa of the urodeum consists of a non-ciliated, stratified, cuboidal to columnar epithelium (Fig. 19). Surface cells have basal round to oval nuclei and appear to be mucus-secreting. The muscularis (Fig. 20) consists of two distinct layers: the inner muscularis (IM), a thin layer of circularly arranged smooth muscles, and the outer muscularis (OM), composed of bundles of longitudinal smooth muscles. The thin serosa (S) is not well defined. More posteriorly, at the copro-urodeal region (Fig. 17c), urodeal glands are less numerous as the cloacal walls become laterally compressed (insert, arrows, Fig. 17c).

3. Histological appearance of the urodeal glands

Urodeal glands are typically branched tubulo-alveolar in structure, containing simple, non-ciliated cuboidal to columnar epithelia, and appear to be mucus secreting. The cells are likely holocrine. The nuclei of the glands are round to oval and occur

basally. Glandular tubules, with round to irregular shapes, empty directly into the lumina of urodeal-limbs or common urodeal chamber (see arrow, Fig. 21).

The terminal parts of the branching collection tubes (secretion units; Fig. 22a) are large and irregularly shaped with low cuboidal epithelia and oval nuclei. These secretion units are primarily seen in the walls of the urodeal limbs and common urodeum. The main collection tubules (Fig. 22b) have high columnar epithelial cells and are usually found to be smaller in diameter, having smaller lumina and being more round than the secretion units. These tubules are most conspicuous in the urodeum walls just before the junction with the proctodeum.

4. Nature of urodeal gland secretions

Secretory products found in the lumina of urodeal glands (Fig. 23) appear as granules and large agranular droplets. The droplets are of an amorphous acidophylic substance and have the following staining properties: in H & E (Fig. 23a), they stain dark pink and in Masson's Trichroom Stain (Fig. 23b), they stain shocking red. With Periodic Acid Schiff (Fig. 24a) and Sudan Black B (Fig. 24b), these smooth droplets show negative staining. Granular products (both small and large granules are apparent) show the following staining properties: in H & E (Fig. 23a), they stained pale pink and are eosinophilic; in Masson's Trichroom Stain (Fig. 23b), they appear light blue. Light blue cytoplasmic bodies are numerous. When stained with Periodic acid Schiff (Fig. 24a), the granular secretion is positive for glycoproteins, but negative for Sudan Black B (Fig. 24b).

5. Seasonal variation in secretory activity

Pre-vitellogenic females (Fig. 25) investigated, had no or very few glandular tubules in the urodeum with no secretory activity observed. In early vitellogenic females (Fig. 26), the epithelial lining bordering the urodeal lumen (UD) displayed relatively small glandular tubules (UG). Although some material was found in the urodeal lumen (possibly secretory material), very little activity was noticed in the glandular tubules themselves. Females in the late vitellogenic (Fig. 27) and early gravid (Fig. 28) stages displayed similar glandular activities: a peak in volume and secretory activity became evident. The two types of tubules (thick-walled and thin-walled; Fig. 28b), are clearly evident in the urodeal glands of females in these two reproductive stages. Females in both mid-gravid (Fig. 29) and late gravid (Fig. 30) stages, showed glandular tubules smaller in volume than during late vitellogenesis or early pregnancy. The lumina of the glandular tubules were considerably smaller than seen in the previous stages, and the epithelium of the urodeal glands were reduced to low columnar, even cuboidal cells. Glandular activity was at its highest in the mid- and late gravid females.

The small, round, thick-walled tubules (Fig. 22b) are probably the collection tubes and the larger thin-walled tubules (Fig. 22a) secretory units found at the terminal parts of these branching collection tubes (Fig. 28).

6. Variation in tubule diameters, epithelial heights and secretion quantities in urodeal glands.

Mean diameters of urodeal gland tubules (Fig. 31) were found to differ significantly among females in different reproductive stages (Nested ANOVA, $F_{5,1892} = 44.625$; $P < 0.0001$). Diameters were small in pre-vitellogenic females, but were significantly increased during early vitellogenesis to mid-pregnancy. Mean tubule diameters were significantly smaller during late pregnancy - comparable to sizes during reproductive inactivity (i.e., during pre-vitellogenesis).

Nested ANOVA indicated that mean epithelial height (Fig. 32) differed among females of different reproductive stages (Nested ANOVA, $F_{5,810} = 96{,}490$; $P < 0.0001$). Mean epithelial height changes paralleled that of tubule diameters in the urodeal glands, having low values during pre- and early vitellogenesis and peaking during late vitellogenic and mid-gravid stages. By late pregnancy, mean epithelial height is significantly smaller than during late vitellogenesis and early pregnancy.

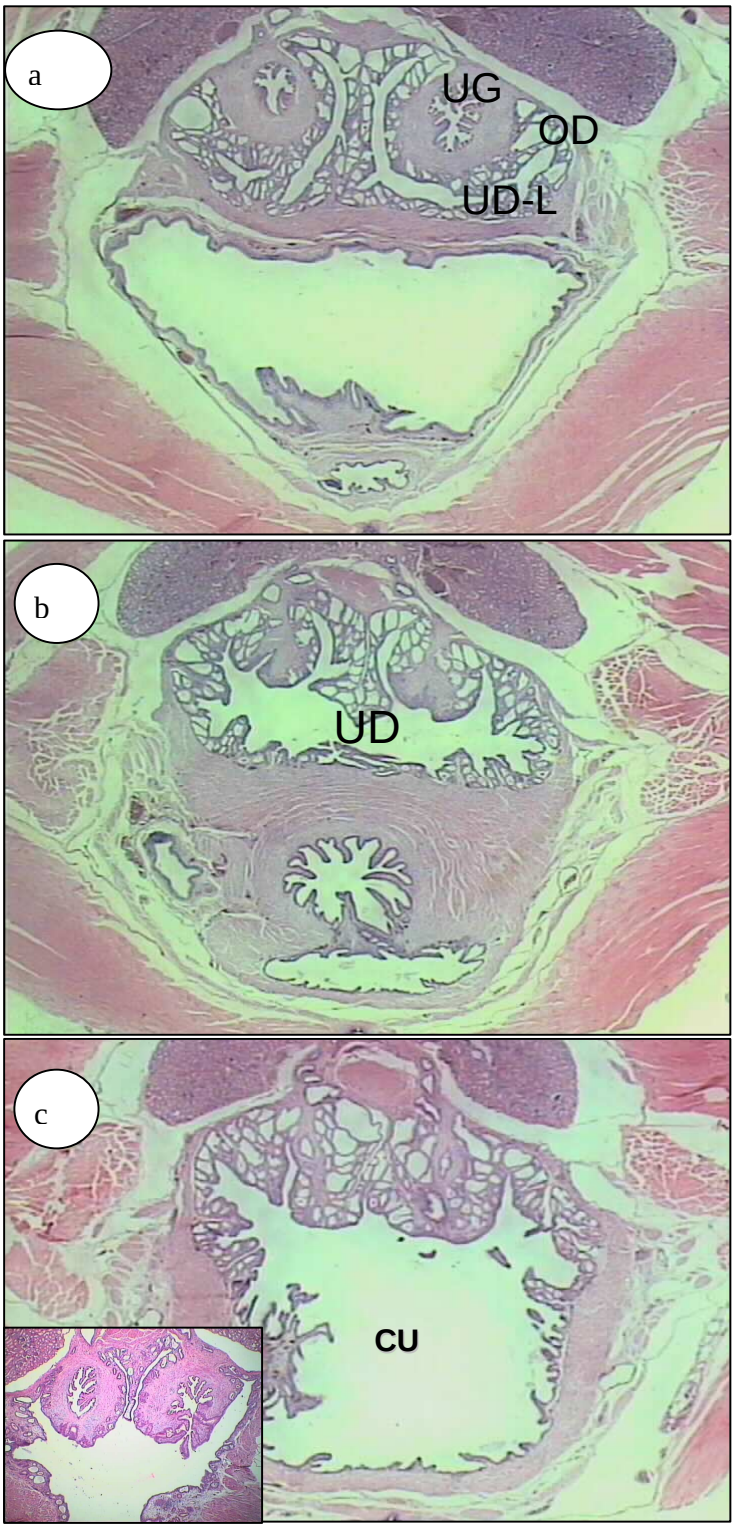

Figure 17. The urodeal region in the cloaca of *Cordylus cataphractus*, illustrating the differences in the urodeal lumen from a) the anterior urodeal limbs (UD-L), b) the intermediate common urodeum (UD) and c) the posterior copro-urodeal (CU) regions. Note the difference in urodeal shapes and the laterally compressed walls (insert) towards the posterior. OD = oviducts; UG = urodeal glands (all at 120x).

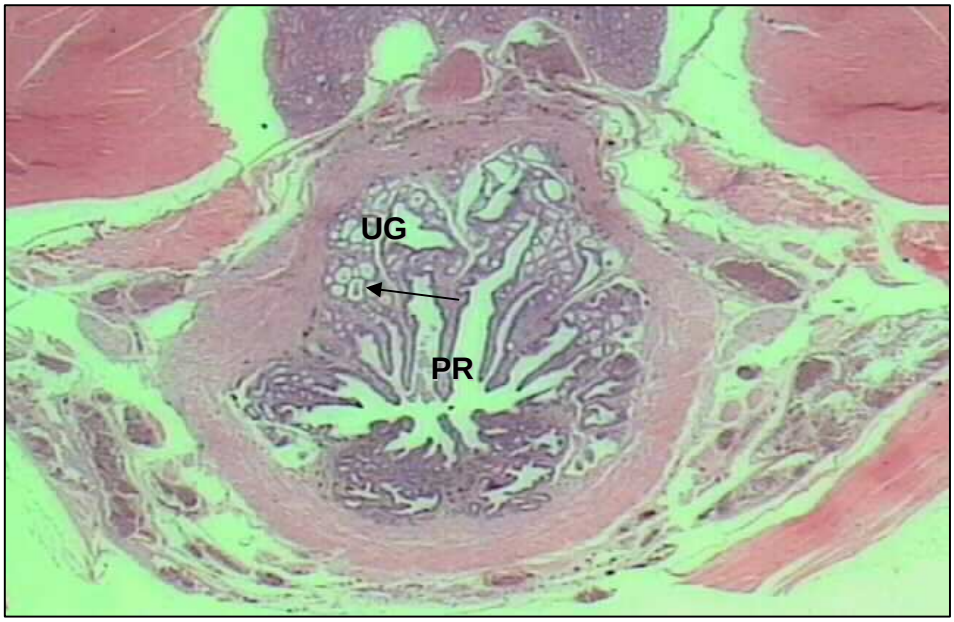

Figure 18. Urodeal glands (UG; see arrows) in a *Cordylus cataphractus* female at the coprodeum-proctodeum (PR) junction (120x).

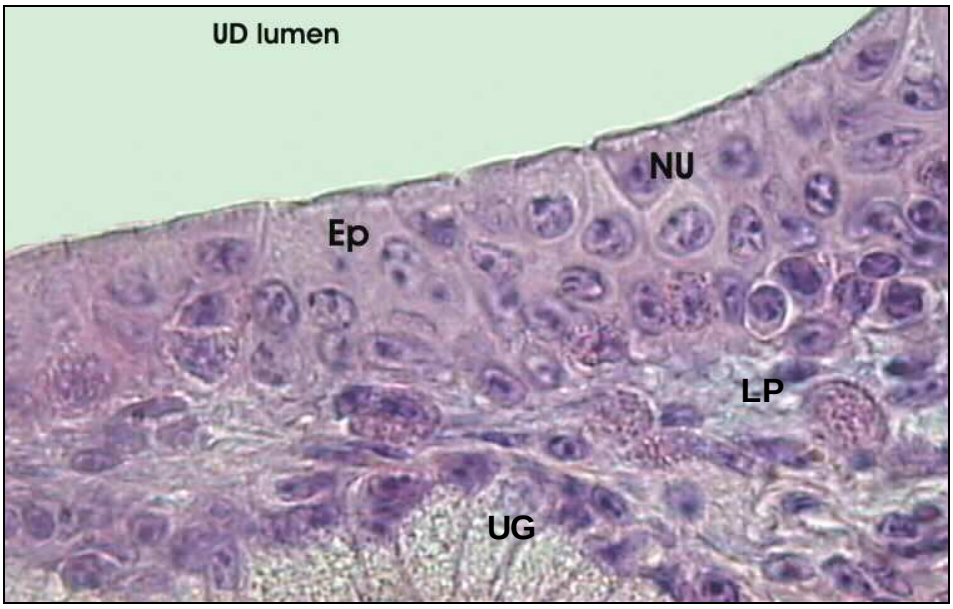

Figure 19. Detail of the stratified epithelium (Ep) of the urodeal limb (UD) of *Cordylus cataphractus* female (1000x). LP = lamina propria; UG = urodeal gland cells.

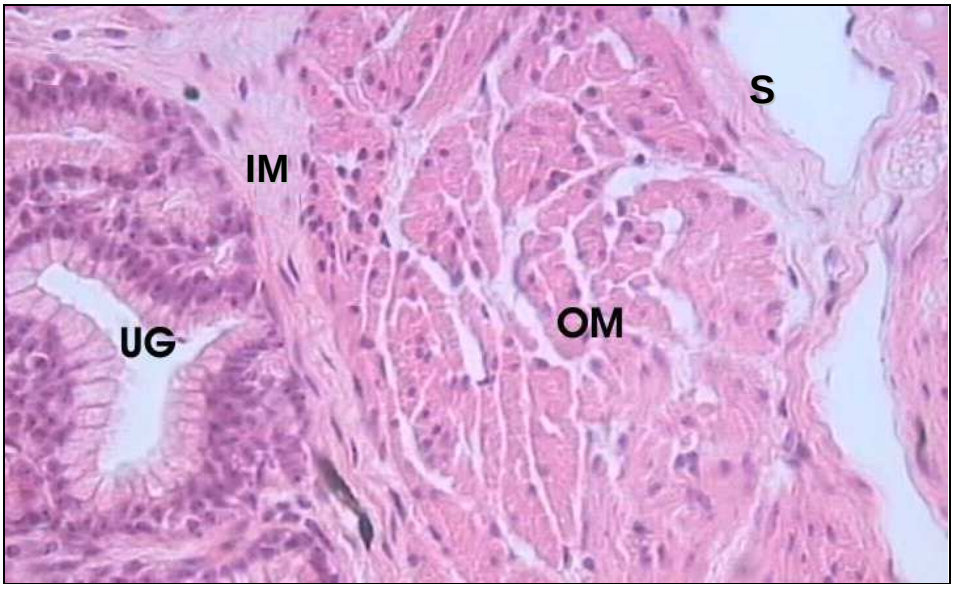

Figure 20. Detail of the urodeal chamber of female *C. cataphractus*, showing the inner and outer layers of muscularis (IM and OM) and serosa (S) of the urodeum. Urodeal glands (UG) are located in the lamina propria (400x).

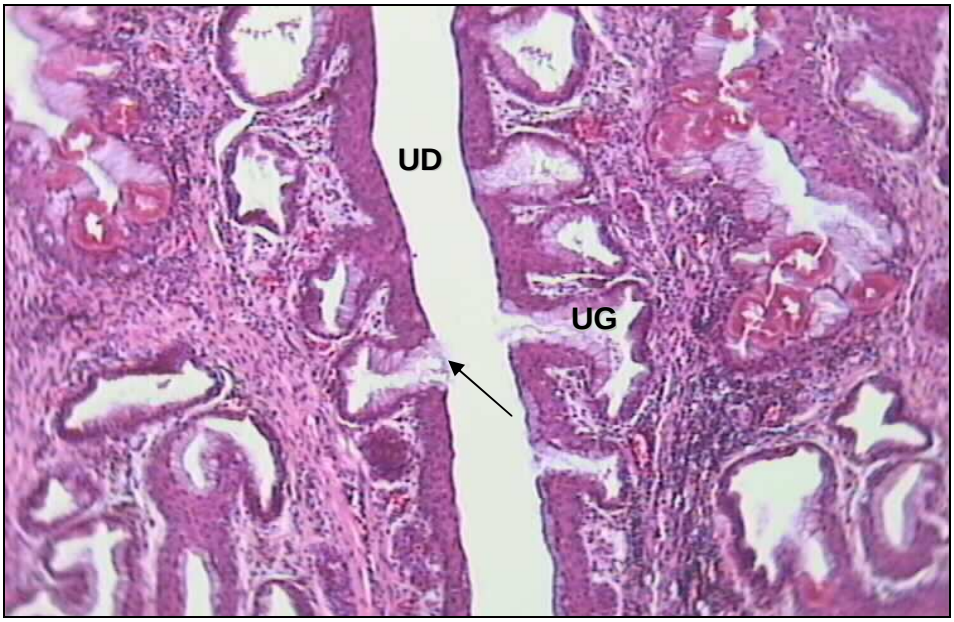

Figure 21. Urodeum of a *Cordylus cataphractus* female, showing glandular tubules of urodeal glands (UG) opening into the urodeal lumen (UD; see arrow) (100x).

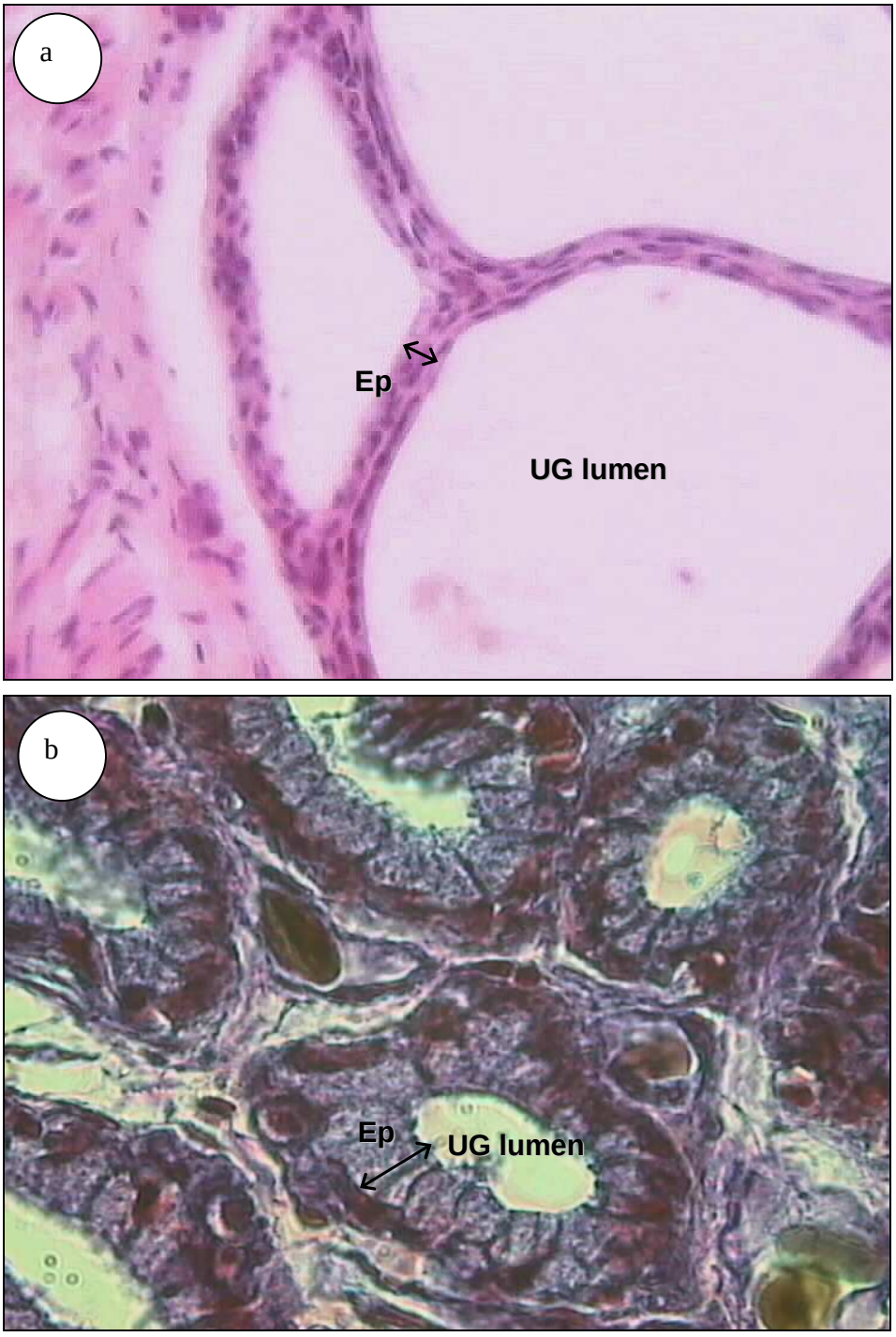

Figure 22. a) Thin walled secretion units of urodeal glands of a *Cordylus cataphractus* female, containing cuboidal to low columnar cells, stained with H & E (400x). b) Note the presence of thick walled collection tubules in the urodeal glands (UG), having tall columnar epithelial cells (Ep), stained with Masson's Trichrome (1000x).

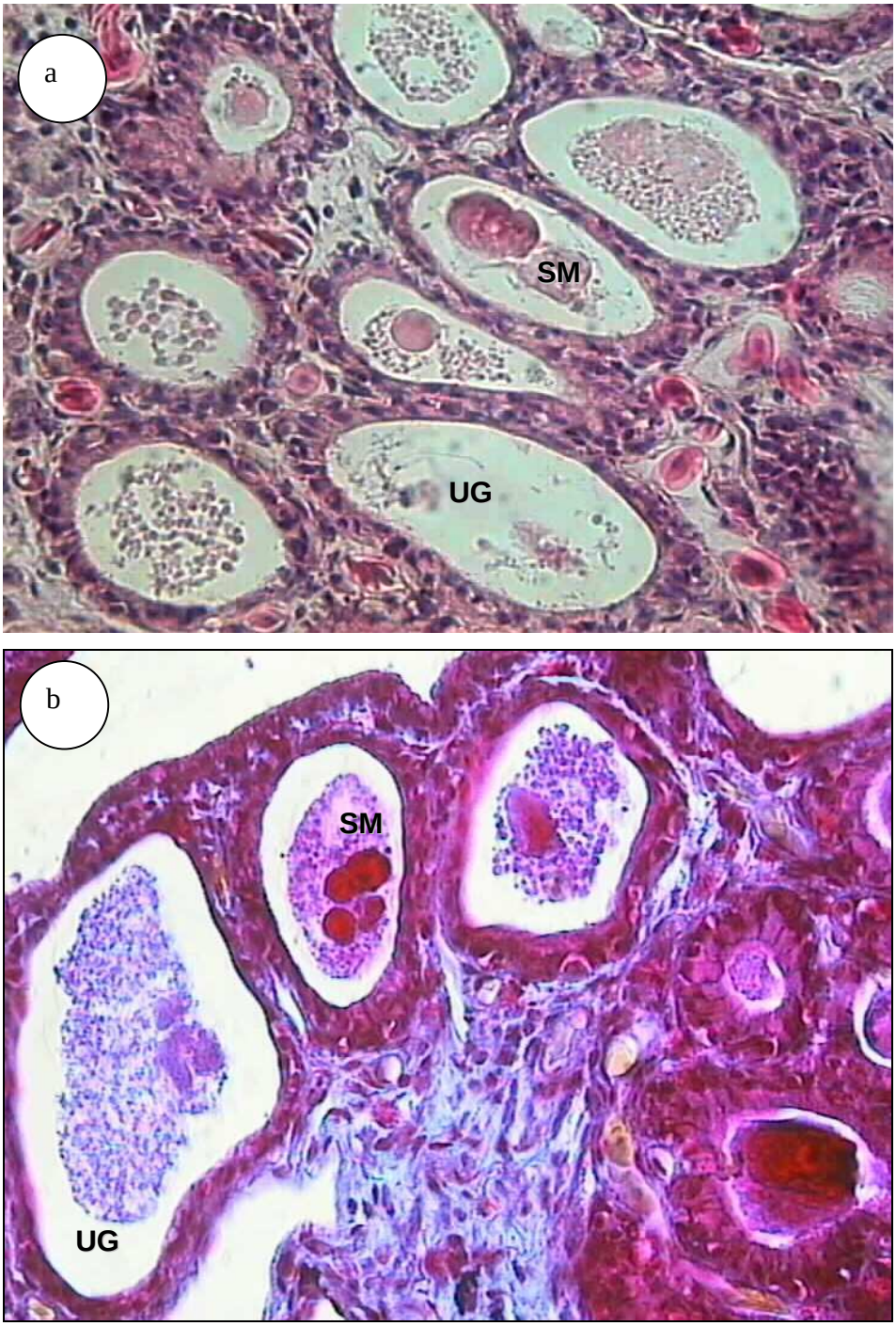

Figure 23. Secretory material (SM) found in urodeal gland (UG) lumina of a late gravid *Cordylus cataphractus* female. Note the two kinds of secretary products, namely granules (both fine and course) and droplets. These were stained with a) H & E; and b) Masson's Trichroom (both 1000x).

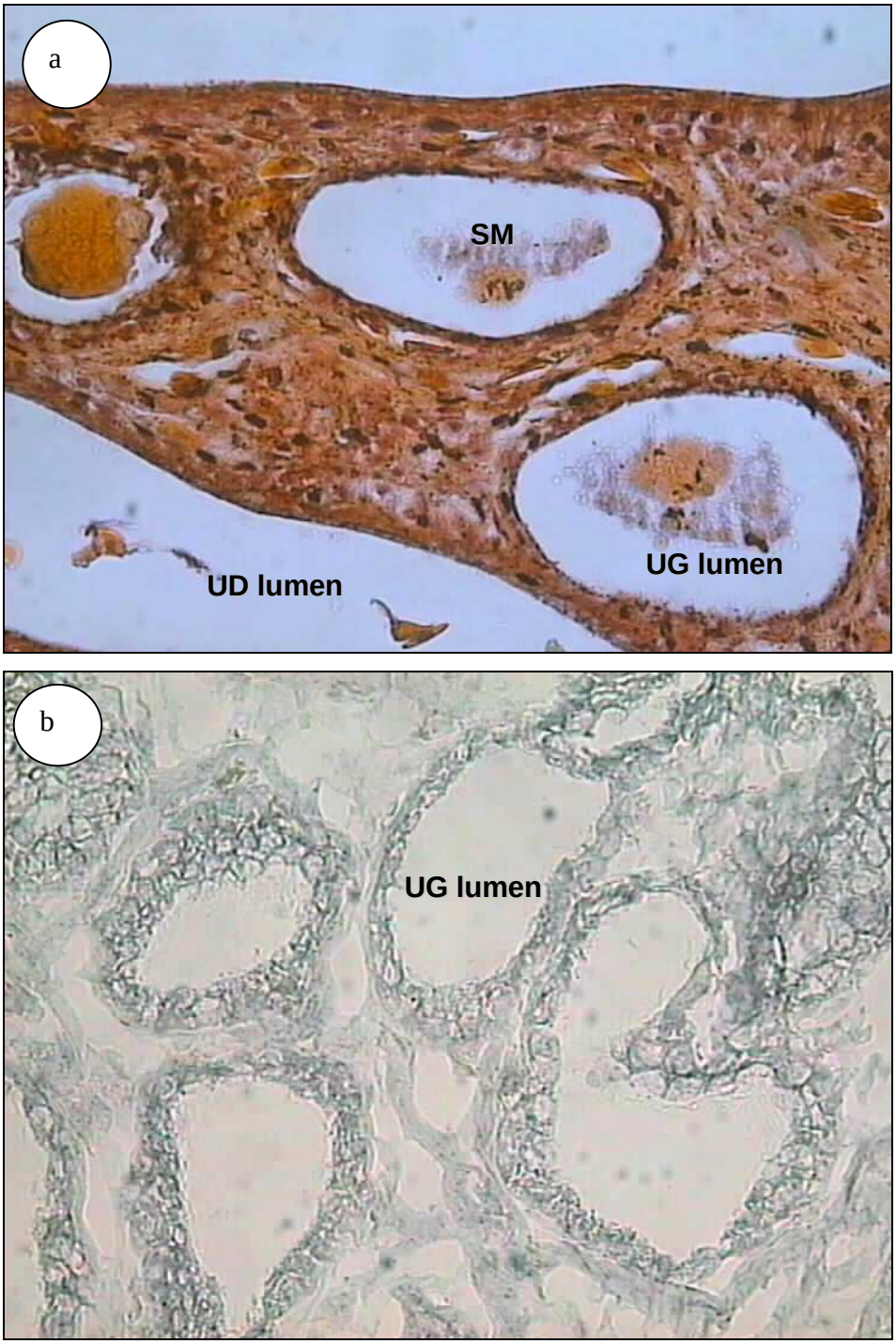

Figure 24. Urodeal glands (UG) of a late gravid *Cordylus cataphractus* female. a) Secretory material (SM) found in the lumina of glandular tubule, stained with PAS (400x). b) No secretory material was found when stained with Sudan Black B (400x). UD = urodeum.

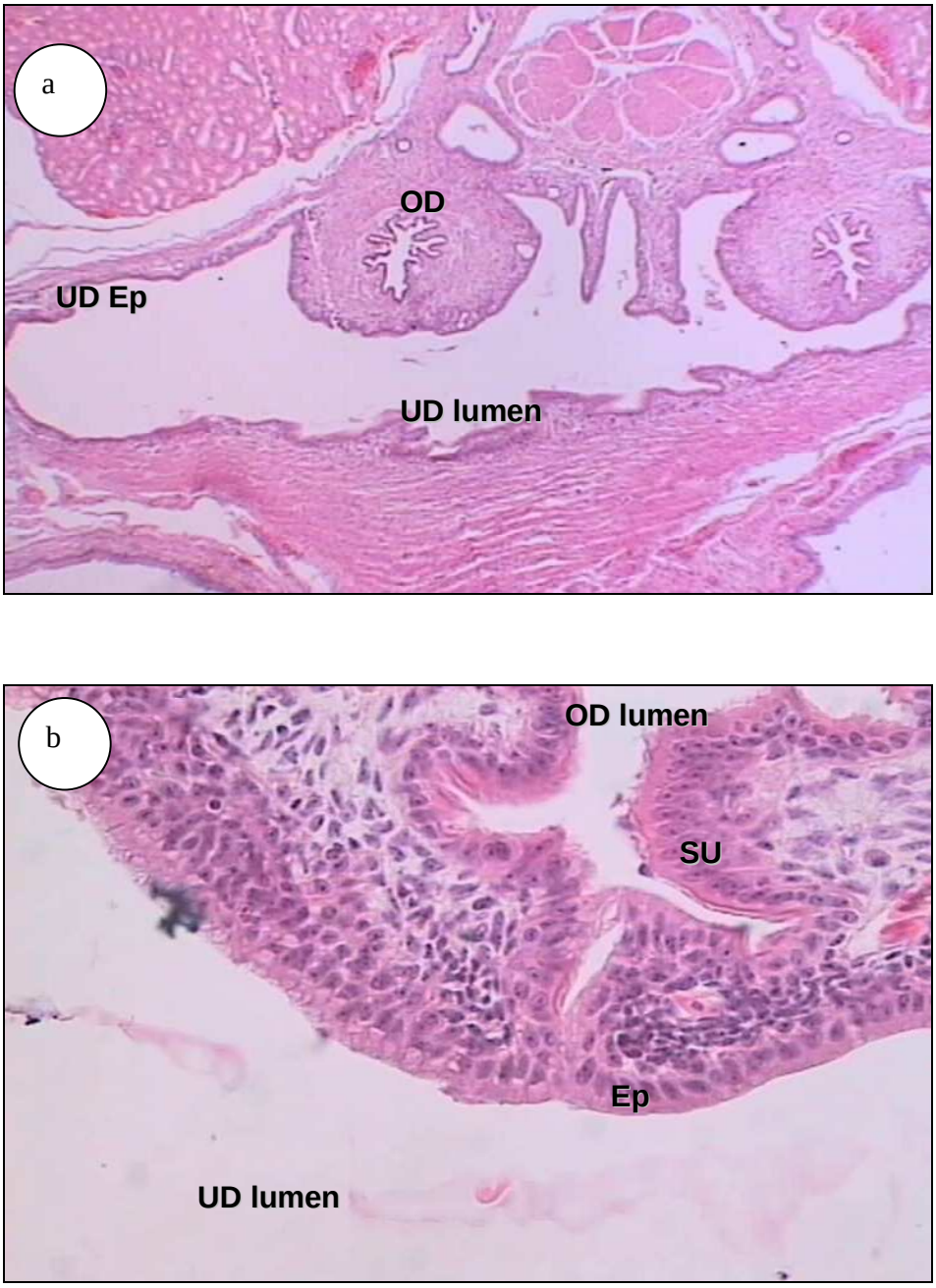

Figure 25. Urodeum (UD) of an adult pre-vitellogenic *Cordylus cataphractus* female. a) No glandular tubules are present, and the urodeal epithelium (UD Ep) appears smooth (40x). b) The oviductal lumen (OD) is small and many leucocytes are seen in the submucosa (SU) (400x).

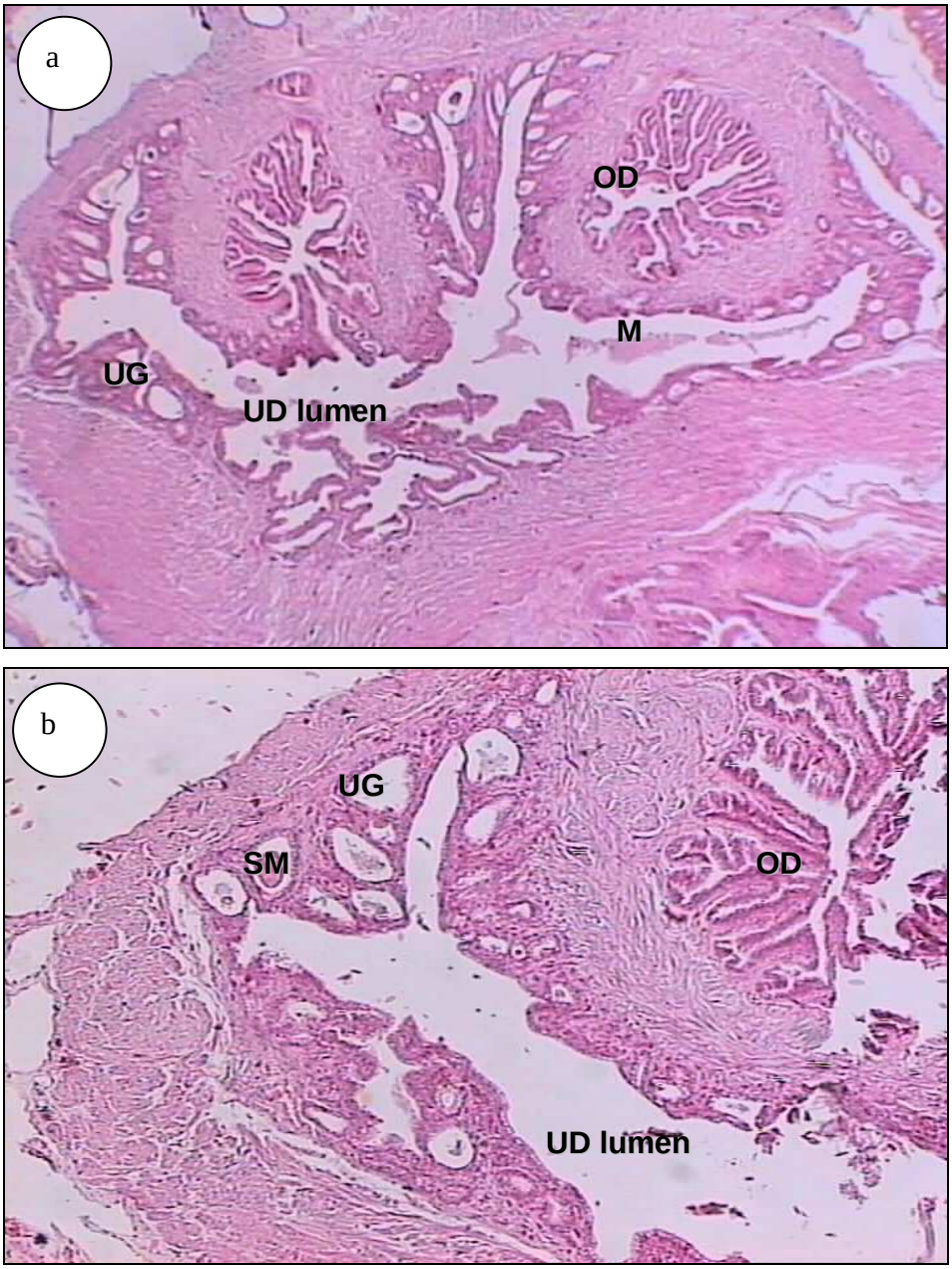

Figure 26. The common urodeum (UD) of an early vitellogenic *Cordylus cataphractus* female, showing urodeal gland (UG) activity. The total volume of the gland is small. a) Material of unknown origin (M) is noticed in the urodeal lumen, this may either be a secretion or artefactual precipitate (40x). b) Some secretory material (SM) is noticed in the glandular tubules. The oviduct (OD) epithelium is highly folded (100x).

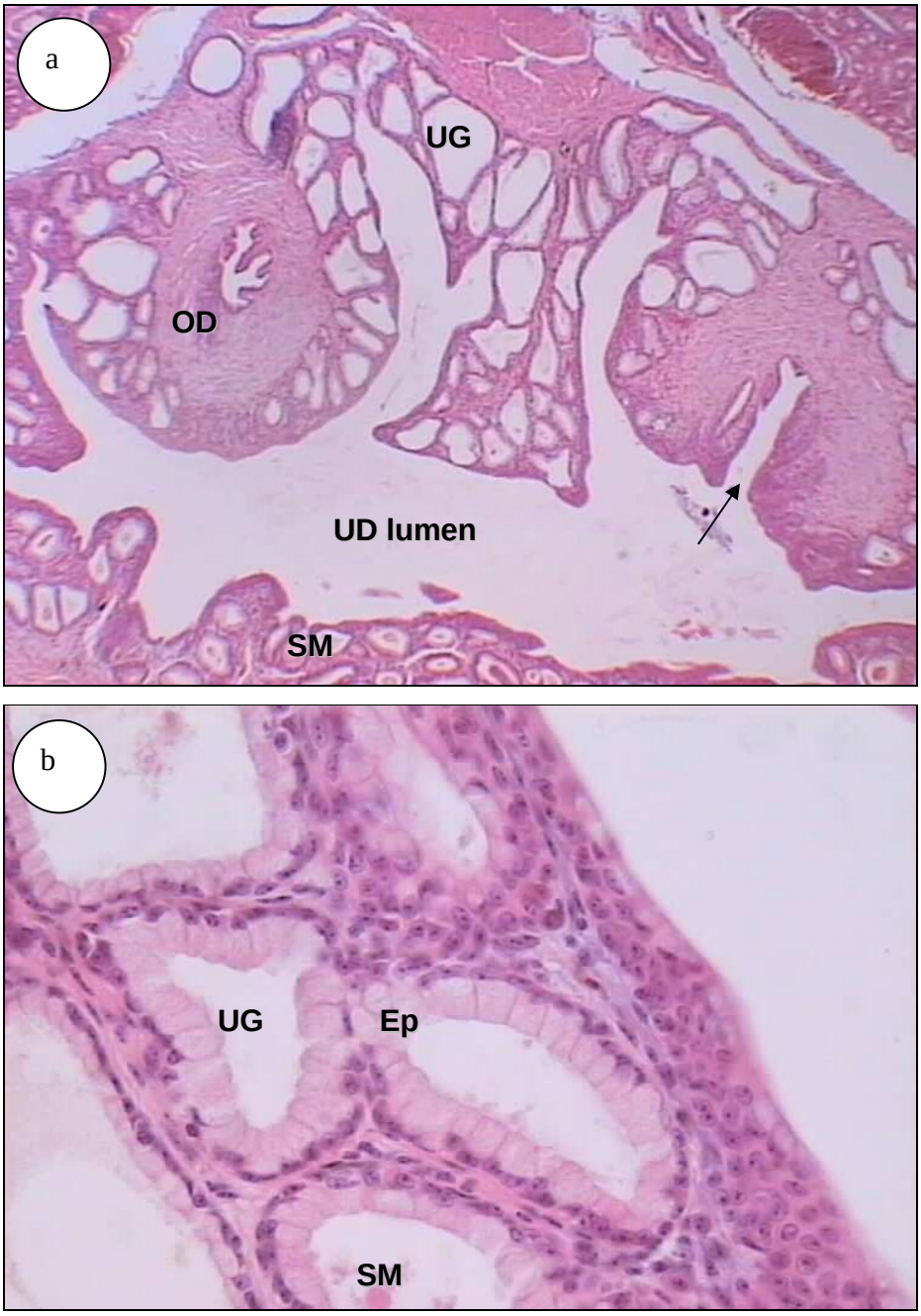

Figure 27. The urodeum (UD) of late vitellogenic *Cordylus cataphractus* female. a) Some secretory material (SM) is observed in the tubules, especially in the smaller ventrally urodeal glands (UG). Note that the oviducts (OD) open into the urodeum (see arrow) (40x). b) Thick walled epithelial walls (Ep) surround the urodeal glands (400x).

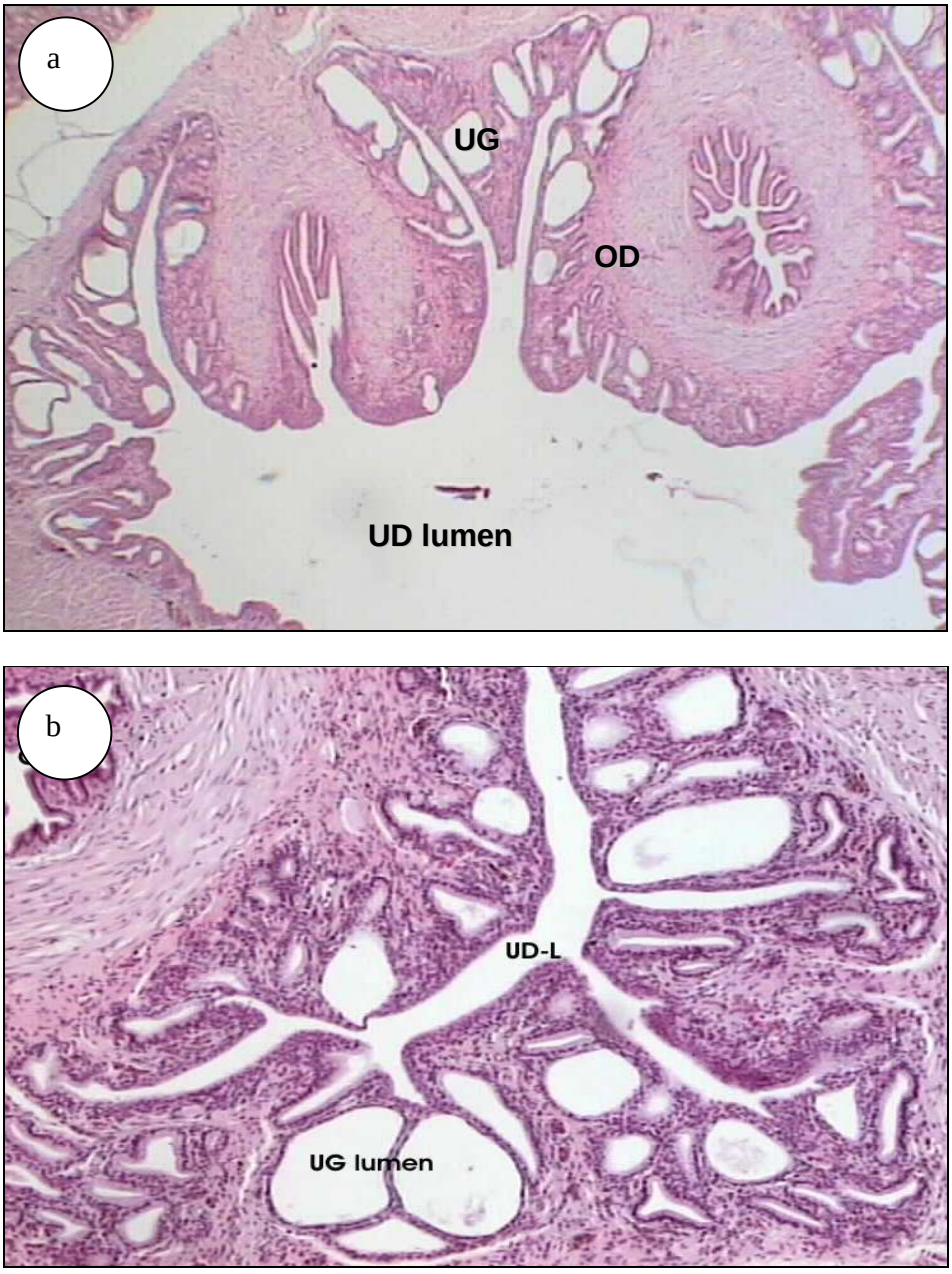

Figure 28. Urodeum (UD) of an early gravid *Cordylus cataphractus* female, showing prominent urodeal glands (UG). The late vitellogenic and early gravid stages appear similar. a) Although the urodeal glands become conspicuous in volume, very little secretory activity is observed in the glandular tubules (40x). b) Note the tubulo-alveolar glandular arrangement in the urodeal limb (UD-L) (100x). OD = oviduct.

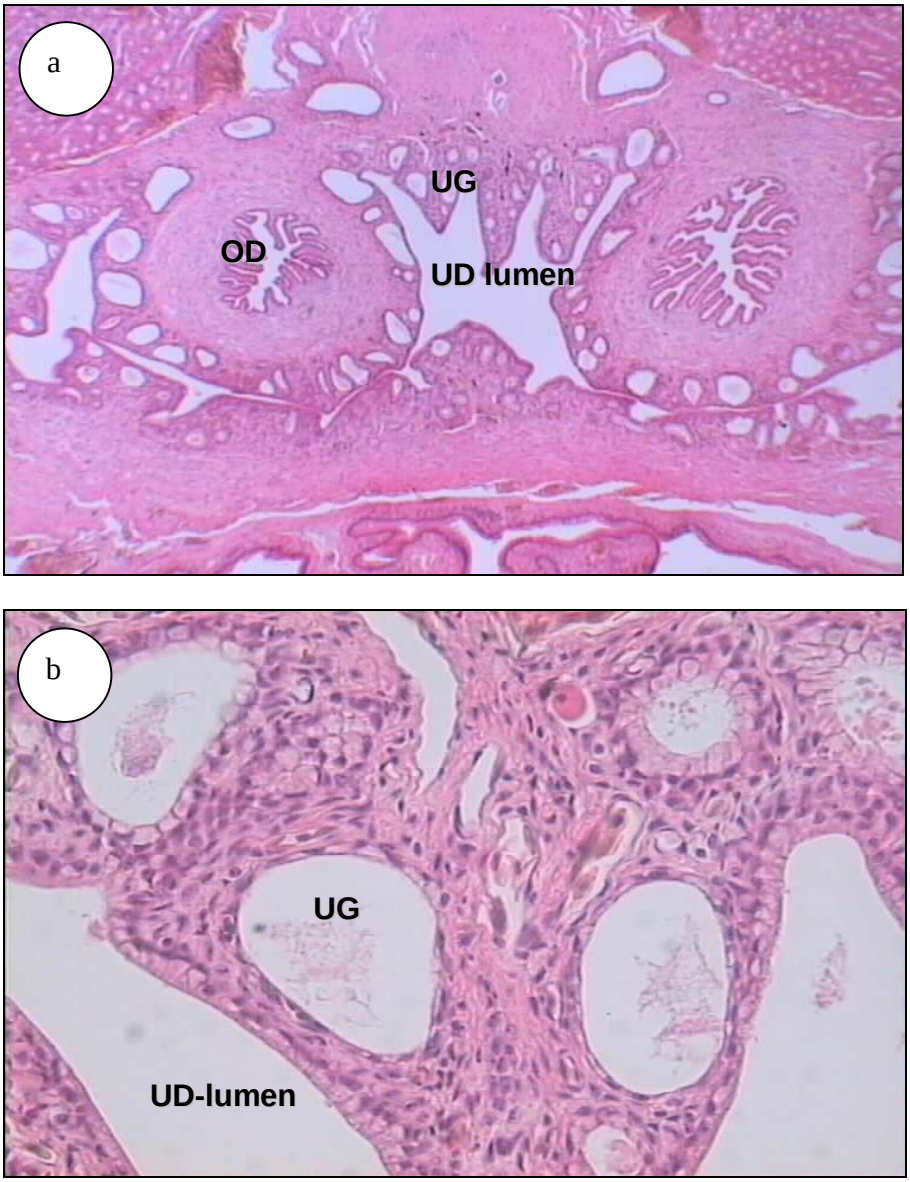

Figure 29. a) Common urodeal (UD) region of a mid-gravid *Cordylus cataphractus* female, showing prominent urodeal glands (UG). These are smaller in volume and have less structure than during late vitellogenic and early gravid stages (40x). b) Thin walled glandular tubules are more prominent than thick walled ones. Secretory activity is significantly higher than in previous stages (400x). OD = oviducts.

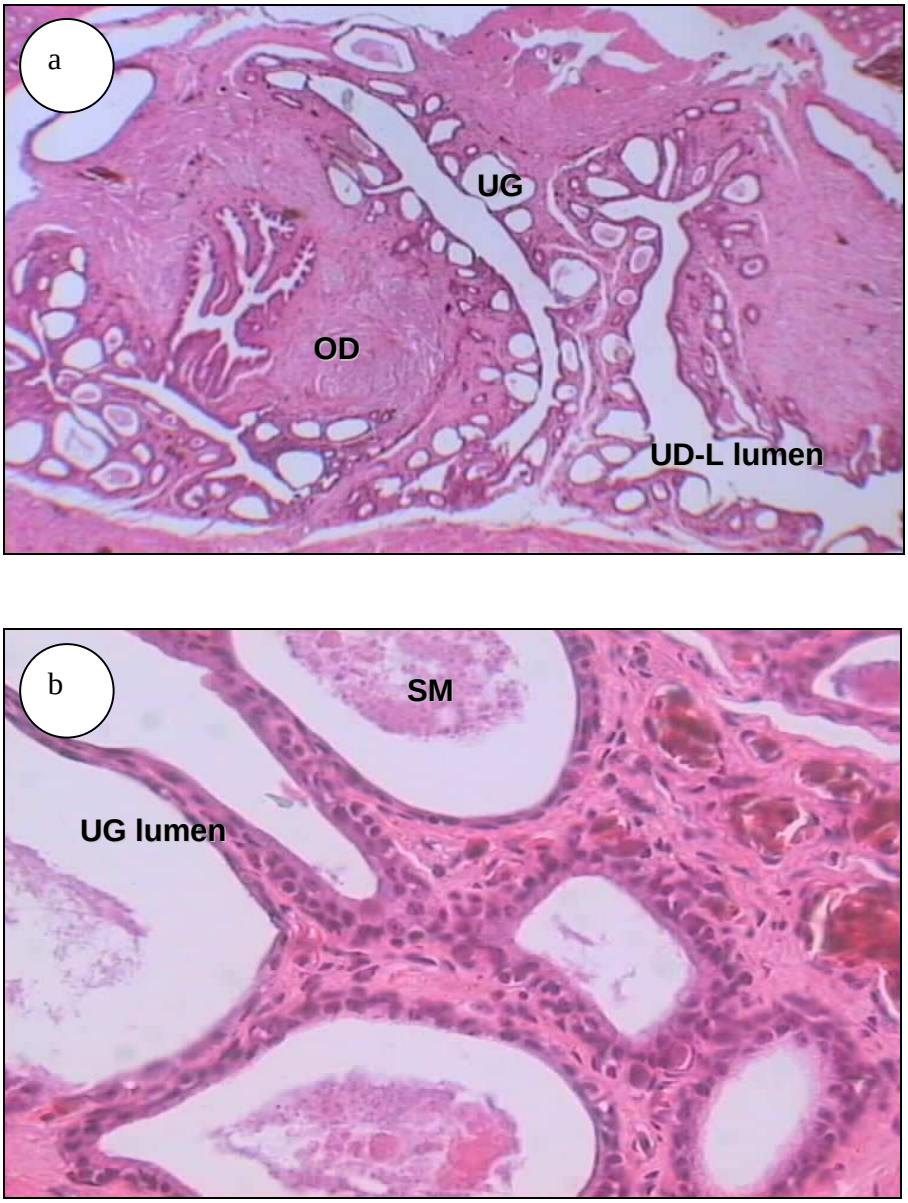

Figure 30. a) Urodeal limb (UD-L) region of a late gravid *Cordylus cataphractus* female. Urodeal glands (UG) are enlarged, although the total volume of the glands is smaller than in late vitellogenic and early pregnant females (40x). b) Note that the thin walled glandular tubules with low cuboidal to columnar epithelial cells are more prominent than thick walled tubules. The nuclei are smaller, more flattened and basal (400x). This reproductive stage is accompanied by peak glandular activity. SM = secretory material.

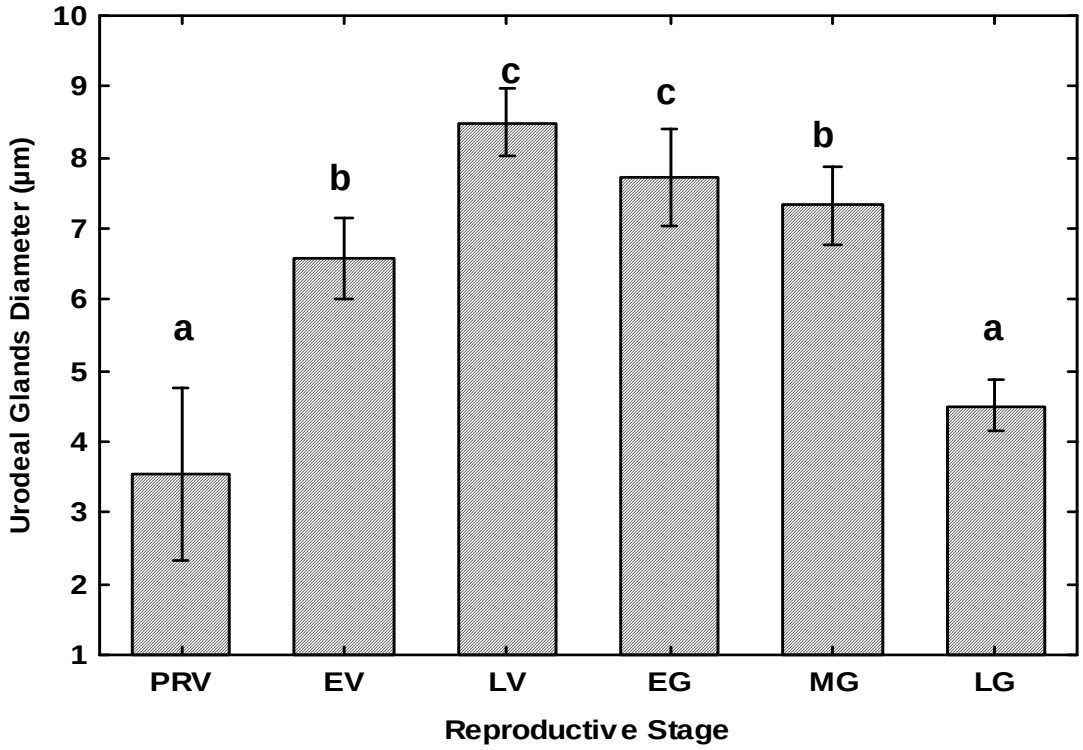

Figure 31. Variation in urodeal gland tubule diameter (means ± 1 standard error) among female *Cordylus cataphractus* of different reproductive stages. Reproductive stages with different symbols are significantly different from each other. Stages are, PRV = Pre-vitellogenic; EV = Early vitellogenic; LV = Late vitellogenic; EG = Early gravid; MG = Mid-gravid; LG = Late gravid.

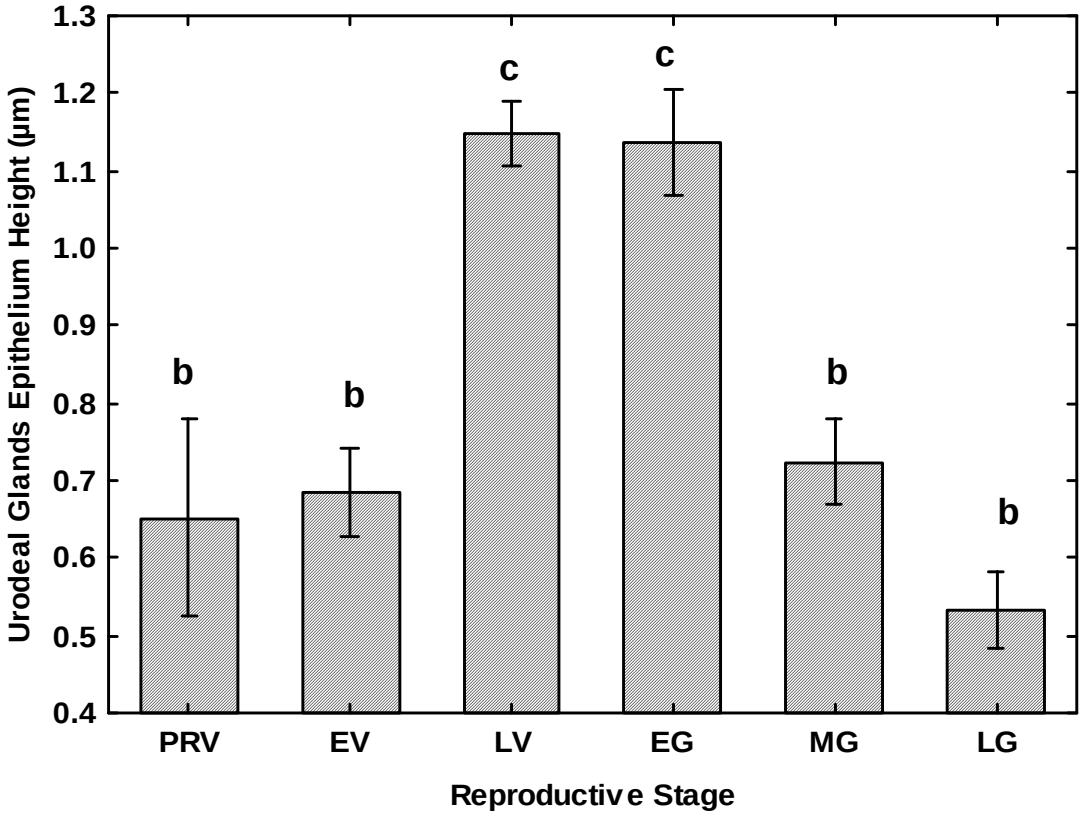

Figure 32. Mean epithelial height variation (means ± 1 standard error) of urodeal gland tubule among female *Cordylus cataphractus* of different reproductive stages. Reproductive stages with different symbols are significantly different from each other. Stages are: PRV = Pre-vitellogenic; EV = Early vitellogenic; LV = Late vitellogenic; EG = Early gravid; MG = Mid-gravid; LG = Late gravid.

– DISCUSSION –

1. Structure of female urodeal glands

The gross anatomy of the female urodeum in *Cordylus cataphractus* concur with that described in other lizards (Gadow, 1887; Regamey, 1935; Withers & O'Shea, 1993). Despite some histological differences, the types of cloacal glands, their distribution, and size in *C. cataphractus* are similar to those reported in other studies, e.g. the lacertid, *Lacerta agilis* (Regamey, 1935), scincid, *Eumeces laticeps* (Trauth *et al*., 1987), gerrhosaurid, *Gerrhosaurus nigrolineatus* (Cooper & Trauth, 1992), and the cordylids, *C. cordylus* and *C. melanotus* (Mackay, 1993).

2. Seasonal variation in urodeal glands

Histological studies reveal that a relationship exists between seasonal variation in the urodeal gland activity and female reproductive cycle in *C. cataphractus*. The major differences between non-reproductive and gravid females are associated with the expansion of the oviducts, the enlargement of the anterior urodeal limbs and an increasing number of urodeal gland tubules. All these changes reflect increases in secretory activity of these glands.

Mackay (1993) reported that urodeal glands were regressed (i.e., having low epithelium height) in gravid females of *C. cordylus* and enlarged (having increased epithelium height) during non-pregnancy. In both *C. cordylus* and *C. melanotus*, there is no apparent difference in secretion quantities when pregnant versus non-pregnant females

is compared. It must be mentioned that he had only three females per species per reproductive stage at his disposal. Secretion quantities may thus vary seasonally in these species, as is the case in *C. cataphractus*.

3. Possible functions of urodeal glands

Seasonal variation in size and secretory activity of the urodeal glands is explicit in *C. cataphractus*, and a clear relationship exists between glandular activity and female reproductive cycle. The glands become enlarged in vitellogenic females and remain active during pregnancy. Peak glandular activity occurs at the time of parturition. Secretory material, especially during the gravid stages, displayed a strong reaction for glycoproteins (PAS +) and muco-substances (H & E). Outside of the breeding season, gland lumina are empty or nearly empty of secretions and histochemical reactions of glandular epithelia were mainly negative for glycoproteins and muco-substances. Regamey (1935) suggested that the urodeal glands in the oviparous *Lacerta agilis* play a role in egg-lying, or may secrete lubrication for fecal and/or reproductive products (Gabe & Saint Girons, 1965) while Mackay (1993) suggested a role in either pregnancy or sperm storage for the viviparous cordylid species he examined. These authors did not consider the possibility that urodeal glands secretions could function as a safety plug preventing bacteria from entering the oviducts, similar to what has been found in mammals where cervical glands produce mucus with antimicrobial properties. A pheromonal function for urodeal glands has been proposed by Whiting (1969), Cooper & Vitt (1986a), Trauth *et*

al. (1987), Cooper (1992) and Cooper & Trauth (1992), but has not being rigorously investigated by experimentation or chemical characterization.

If the function is pheromonal (e.g. for onset of courtship; attraction of males; sex/ or species recognition), it is expected that secretory activity will peak when the gland conveys information on sexual or social status during the mating season in late spring to early summer. Maximal spermatogenesis then occurs in males and females are in their early to late vitellogenic reproductive stages (Flemming & Mouton, 2002). The present study reveals that during this period low glandular activity is present. Whether or not these glands produce the chemical cues used in sex and species recognition, however, remains to be clarified through appropriate experimentation. If the function of these glands is related to social communication in *C. cataphractus* (especially in view of their group-living behaviour), it is expected that glandular activity will be present throughout the year. As little glandular activity occurs specifically during the pre-vitellogenic reproductive stage in autumn and very little during the early vitellogenic stage, such a role seems unlikely. If the function or urodeal glands in *C. cataphractus* is mechanical on the other hand (such as lubrication during birth, or acting as a plug to seal oviducts), it is expected that glandular activity would be seasonal, increasing from the start of pregnancy in summer and peak around parturition in autumn and that large volumes of mucoid secretions would be produced. It was found that peak glandular activity occurred during mid- to late pregnancy and this correlation between the peak glandular activity and gravidity suggests some function related to pregnancy.

To conclude - in view of the fact that urodeal glands are only present in females and seasonal variation in glandular activity and structure is evident, it is likely that urodeal glands have a lubrication function in *C. cataphractus* females. Specifically, it is probably related to late pregnancy or birth, rather than being actively involved in pheromonal communication.

CHAPTER 4: STRUCTURE AND SECRETORY ACTIVITY OF PROCTODEAL GLANDS IN *CORDYLUS CATAPHRACTUS*

- INTRODUCTION -

The lizard cloaca houses two kinds of exocrine glands associated with the urodeal and proctodeal compartments. Those found in the latter, are generally called dorsal and ventral glands in lizards although they appear similar in structure and may just be dorsal and ventral aspects of the same gland (Trauth *et al*. 1992; Mackay 1993; Cooper *et al.* 1996). The term proctodeal glands may be of more practical use, as the 'ventral glands' are not always present. Indeed, glands of the proctodeum have been called all kinds of names, especially in the earlier literature (e.g., glande vestibulliene (Lerebouillet, 1851), anal glands (Gadow, 1887), phallusdrüse (Utterhössel, 1902), kloakendrüse (Beuchelt 1936)), but the current use of dorsal and ventral glands is expected to persist in the mainstream literature for some time.

In most lizards studied to date, the dorsal and ventral gland masses are paired and clusters are located in the dorso-lateral and ventro-lateral walls of the proctodeum. Where dorsal and ventral masses join, a distinct glandular ring is observed around the proctodeal lumen in cross-section, this being the situation found in several lizard species belonging to the families Agamidae, Chamaeleonidae, Gekkonidae, Lacertidae, Scincidae, Gerrhosauridae, Cordylidae and Xanthusiidae (Unterhössel, 1902; Gabe & Saint Girons, 1965; Whiting, 1969; Trauth *et al.,* 1987; Cooper & Trauth, 1992; Mackay, 1993).

Although, exceptions are found, for instance, Cooper & Trauth (1992) reported that proctodeal glands in *Gerrhosaurus nigrolineatus* females are thinly scattered, but males have large and conspicuous ones. Gekkonids and helodermatids typically have four dorsal gland clusters (Whiting, 1969). Ventral glands also vary much among lizard species, having been described as absent in *Eumeces laticeps* (Trauth *et al.*, 1987), thinly scattered in *G. nigrolineatus* (Cooper & Trauth, 1992), unpaired in *Acontias meleagris* (Van der Merwe, 1944), occurring as glandular 'fields' in *Lacerta agilis* (Wöpke, 1931) or more than two glandular clusters masses may be present. In gekkonids and helodermatids, for example, ventral gland masses are divided into two, four six or eight distinct clusters (Whiting, 1969).

Proctodeal glands may be branched-tubular (e.g., in gerrhosaurids and cordylids (Cooper & Trauth, 1992; Mackay, 1993)), tubulo-alveolar (e.g., in scincids (Trauth *et al.*, 1987)), or compound-alveolar (e.g., in teiids (Hardy & Cole, 1981)) in structure. Gabe & Saint Girons (1965) reported that the distribution of serous and mucous cells in the proctodeal glands varies among species, and often between sexes of the same species. Males of many gekkonids, iguanids, scincids and xanthusids have both mucous and serous cell types in their proctodeal glands, while females have only mucous cells (Gabe & Saint Girons, 1965; Whiting, 1969). Mackay (1993) observed mucous cells in proctodeal glands of both sexes, but he stated that serous cells may only be present in some areas of male cordylids. Gabe & Saint Girons (1965) reported a tendency towards the concentration of mucous cells in the ventral glands and serous cells in the dorsal glands of scincids and gekkonids.

Many lizards show seasonal variation in aspects of cloacal histology and this is generally correlated with the reproductive cycle (Gabe & Saint Girons, 1965; Trauth *et al.*, 1987). Such variation is also evident in urodeal glands, but proctodeal glands of both sexes in all lizards studied to date undergo no or very little seasonal variation in size and secretory activity (Regamey, 1935; Trauth *et al.*, 1987; Cooper & Trauth, 1992; Mackay, 1993). Functions of these glands in lizards are poorly understood. A number of studies reporting on histological or histochemical investigations of these glands suggest that proctodeal glands are involved in lubrication and/or pheromonal communication (Regamey, 1935; Cooper *et al.*, 1986; Trauth *et al.*, 1987; Cooper & Trauth, 1992; Mackay 1993).

The Armadillo Lizard, *Cordylus cataphractus*, is a cordylid species suitable for studying proctodeal gland histology and function for two reasons. Firstly, detailed histological observations exist for two congenerics (i.e., those studied by Mackay, 1993). Second, much is known about the life history of this species (Peers, 1930; Branch, 1988; Mouton *et al.*, 1999; Visagie, 2001; Flemming & Mouton, 2002).

In the present study, I followed three routes for determining whether changes in proctodeal glands of *C. cataphractus* are seasonal or not, what the nature of proctodeal gland secretions are, and finally, the possible functions of these secretions. Firstly, I compared histological appearance of glands in lizards of different reproductive stages. This is the favoured method mentioned in the literature, but often only two or three stages were compared and sample sizes were normally very small. Secondly, I measured epithelial heights and tubule diameters of proctodeal glands and compare these among males and females in different reproductive stages. Studies on lizard proctodeal glands

mention differences in extent of glands and epithelial heights at different times of the year, but these are qualitative statements, unsubstantiated by morphometric analyses. My last approach was to use histochemistry to determine the nature of the secretions, and then to deduce a possible function (lubrication and/or pheromonal communication) from it. Very few studies reporting on proctodeal gland structure and function in lizards have taken the latter approach.

Regarding possible function(s) of proctodeal glands, I made the following predictions: If it is for lubrication, one would expect that large volumes of mucoid secretion are formed in the glandular tubules, that the glands will be absent or inactive in one sex if it was related to a sex specific reproductive event, that secretory activity will be seasonal if the function relates to a seasonally occurring event, or that the secretory activity will be continuous if the function relates to events occurring frequently. If it is for pheromonal communication, one would expect that the secretion will include lipoidal components, that the glands will be absent or inactive in one sex if they were conveying sex-related information, that the secretory activity will be seasonal if the glands convey information on sexual or social status, or that secretory activity will be continuous if the function relates to events occurring frequently.

- MATERIALS AND METHODS -

1. Materials

Specimens were collected in the Lamberts Bay-Graafwater district and accessioned into the Ellerman Collection of Stellenbosch University (see Chapter 2 for details). The cloacal complexes of 22 adult *Cordylus cataphractus*, 18 females and four males, were excised and histologically examined for the structure and activity of proctodeal glands. Female specimens were divided into six reproductive groups as described in Chapter 3 (see Table 3). At least one male specimen per group was sampled, representing four reproductive stages (Table 4) as determined from presence or absence of spermatozoa and size of the testes (established by Flemming & Mouton (2002)). The inactive stage (stage 1) represents minimum testes volume and spermatogenic regression. Stage 2 represents onset of spermatogenesis, while Stage 3 represents maximum spermiogenesis and Stage 4 were sampled when mating occurs (see Appendix 2 for more detail on specimens used as well as collection- and locality data).

2. Histological preparation of tissue samples

Standard histological procedures as described in Chapter 2 were followed. Serial sections were prepared for at least one specimen per reproductive stage. Routine sections were made in the proctodeal region of the other specimens. Slides were stained with Erlich's Haematoxylin and Eosin (H & E) for general cytological examination, Masson's

Trichrome Stain was used for presence of collagen and mucoid substances, and Periodic Acid Schiff (PAS) for presence of glycoproteins. The cloaca of a single mid-gravid female was excised and fixed in formalin. Routine sections were made at 8 µm with a cryostat and slides were stained for presence of lipids using Sudan Black B.

3. Statistical analyses

Diameters and epithelial heights of cross sections of female proctodeal gland tubules were obtained to the nearest 0.001 mm using a compound microscope and Leica Quips Image Analysing Software. Ten diameters and 10 epithelial heights of these glands were taken in the anterior as well as posterior regions of both aspects (dorsal and ventral) of the glands. Nested ANOVA (with the region and individuals as independent factors) was used to test for differences in diameters and epithelial heights among reproductive stages, and $P < 0.05$ was considered significant.

Table 3. Number of female *Cordylus cataphractus* individuals at different reproductive stages used for histological evaluation of cloacal gland presence and activity.

Reproduction status	Collection month	Sample size (n)
Pre-vitellogenic	Jan to Apr	3
Early vitellogenic	May to Jul	3
Late vitellogenic	Apr to Oct	3
Early gravid	Oct to Jan	3
Mid-gravid	Jan to Mar	3
Late gravid	Mar to Apr	3

Table 4: Sample sizes and reproductive stages of *Cordylus cataphractus* males examined.

Reproduction status	Collection month	Sample size (n)
Inactive males	Dec	1
Early spermatogenesis	Oct	1
Peak spermatogenesis	Oct	1
During mating	Oct	1

– RESULTS –

1. Gross anatomy of the proctodeum and its glands

The gross anatomy of the cloacal complex in *Cordylus cataphractus* is described in detail in Chapter 2. The boundary between the urodeum and short proctodeum is indicated by a thick circular muscle grouping in most specimens studied (Fig. 33). The anterior and middle regions of the proctodeum have a round lumen with medium to high mucosal folds (Figs. 34 & 35), becoming dorso-laterally flattened towards the transverse vent (see Figs. 36, 37, 38). Gland masses are found in the ventral and dorsal walls of the proctodeum of both sexes of this species, being larger in male individuals than in females studied. These exocrine proctodeal glands are usually also more prominent ventrally than dorsally (Fig. 39a, b).

2. Micro-anatomy of the proctodeum and position of its glands

The mucosa of the anterior proctodeal region in both sexes consists of a pseudostratified columnar epithelium and these cells are clearly mucoidal (see insert, Fig. 33). The mucosa changes posteriorly into a stratified squamous epithelium (Fig. 38) and becomes cornified continuous with the external skin at the vent.

Distinct masses of compound tubulo-alveolar proctodeal glands occur in the thick lamina propria of the proctodeum. Tubules appear to open towards the posterior region of the proctodeum. Dorsal and ventral aspects of the proctodeal glands appear similar in

basic structure and cell types, but differ in their relative positions above and below the proctodeal lumen. In about half of the 16 specimens examined, the ventral masses lie more anterior than the dorsal masses (see insert, Fig. 34). The dorsal and ventral glandular masses form distinct clusters, essentially two dorso-lateral and two ventro-lateral ones (see arrows, Fig. 35). Where dorsal and ventral masses join in the anterior-posterior plane, these clusters in cross-section appear to form a "ring" (Fig. 36), surrounding the proctodeal mucosa. In some individuals, these glands form a U-shaped arrangement (see insert, Fig. 36) as a medial muscle layer extends between the dorso-lateral clusters before they form a ring more posteriorly.

In the anterior region of the proctodeum, a relatively thick inner muscularis lies exterior to the lamina propria. In most individuals, the inner muscularis appears thicker ventrally than dorsally. Some longitudinal smooth muscles of the outer muscularis occur in the ventral wall of the proctodeum. Also evident in this region, is a blood plexus (BP; Fig. 36) lateral to each side of the proctodeum. The inner muscularis becomes reduced towards the middle of the coprodeum (Fig. 37) and disappears towards the vent (Fig. 38), it also reduces faster in thickness ventrally than dorsally.

3. Histology of the proctodeal glands

Lumina of the proctodeal gland tubules appear to be more irregular in shape, and their diameters to be larger (Fig 40) when compared to urodeal glands (see Fig. 23, Chapter 3). Tubules of the dorsal gland masses empty into the postero-dorsal wall of the proctodeum, while the tubules from the ventral gland masses empty into the ventro-

caudal part. Glandular tubules are lined with simple cuboidal epithelium and the glandular masses are surrounded by loose connective tissue.

4. Nature of proctodeal gland secretions

Both serous and mucus-secreting cells are present in proctodeal glands of *Cordylus cataphractus* males and females, but the former predominate. Secretory activity in males appears to be more intense as glands are larger in extent. Secretion granules are conspicuous in apices of epithelial cells, and luminal secretory material appears dense and fine granular (Fig. 41). This contrasts with urodeal gland secretions described in Chapter 3 in which fine and course granular material, as well as large droplets are found. Proctodeal gland secretion granules stain dark pink with H & E, showing an acidophilic reaction, and appear clear pink when stained with Masson's Trichrome (Fig. 42). Both epithelia and secretory material stain positively with PAS (Fig. 43), but negatively with Sudan Black B (Fig. 44). The mentioned histochemical staining profile suggests that proctodeal gland secretions are rich in muco- or glucoproteins, with little or no lipids present.

5. Variation in tubule diameters, epithelial heights and secretion quantities in proctodeal glands

Nested ANOVA indicated that mean diameters of proctodeal gland tubules do not differ significantly among females of different reproductive stages ($F_{5,432} = 1.8216$; $P = 0.1073$) (Fig. 45). Mean epithelial heights of female glands also showed no significant

seasonal variation (Nested ANOVA, $F_{5.192} = 2.1225$; $P = 0.06443$) (Fig.46). Mean diameters and epithelial heights of male proctodeal glands were not statistically compared because of too low sample sizes.

Males and females of all reproductive stages had secretory material in the lumina of proctodeal glands, and there was no evidence of sex dependent differences. Variation in absolute secretion quantities among individuals seems evident, but was not evaluated statistically.

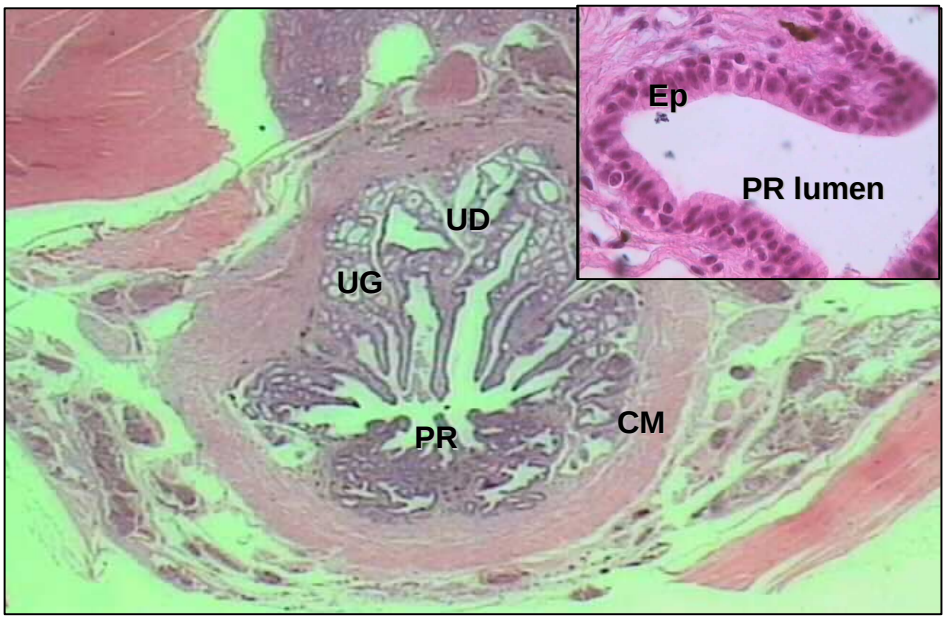

Figure 33. The proctodeum (PR) of a *Cordylus cataphractus* female at its junction with the urodeum (UD). Note the presence of urodeal glands (UG) in the dorsal wall of the urodeum (120x). The proctodeal epithelium (insert) consists of mucus secreting cells (400x). CM = circularly arranged smooth muscles.

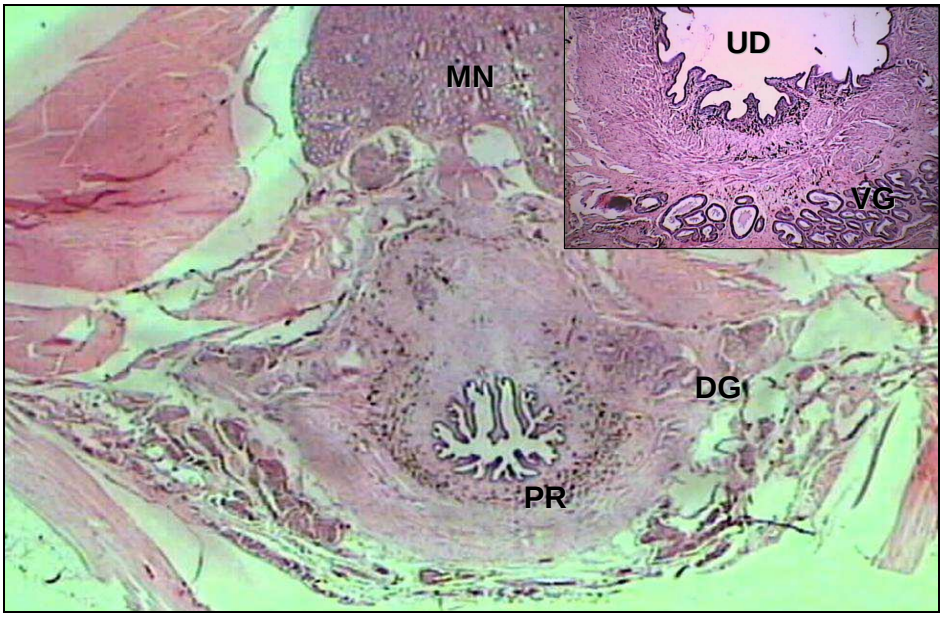

Figure 34. Anterior region of the proctodeum (PR) of a late vitellogenic *Cordylus cataphractus* female. Dorsal glands (DG) are present lateral to the proctodeum lumen. MN = Metanephros (120x). The insert shows a specimen (in this case an early vitellogenic female) in which ventral glands (VG) are seen before dorsal ones in the anterior proctodeum. Note that the lumen is still that of the urodeum (UD) (100x).

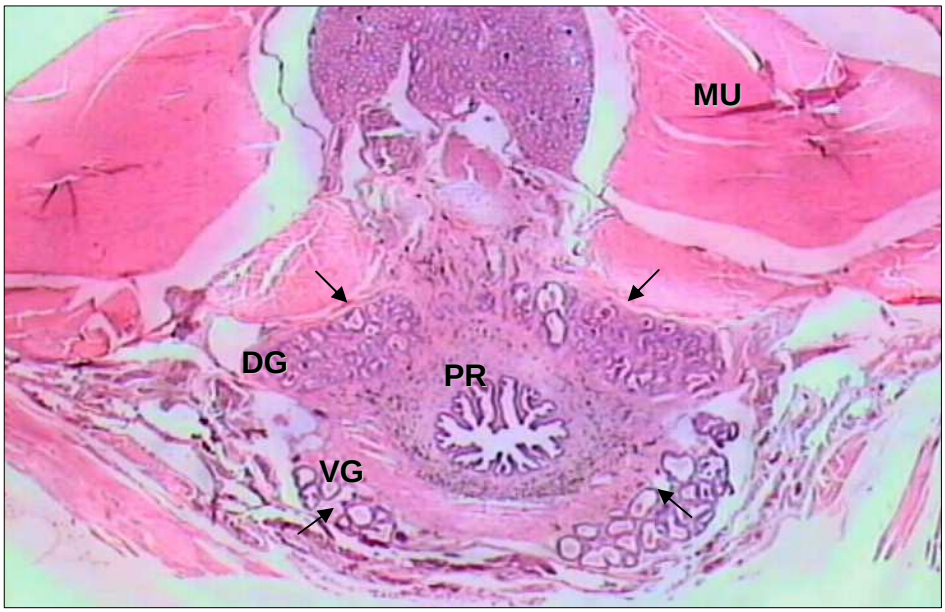

Figure 35. Proctodeum (PR) of a *Cordylus cataphractus* female. Dorsal (DG) and ventral (VG) glandular masses are present, expressed as four distinct clusters (see arrows) around the proctodeal lumen (120x). MU = Skeletal muscle.

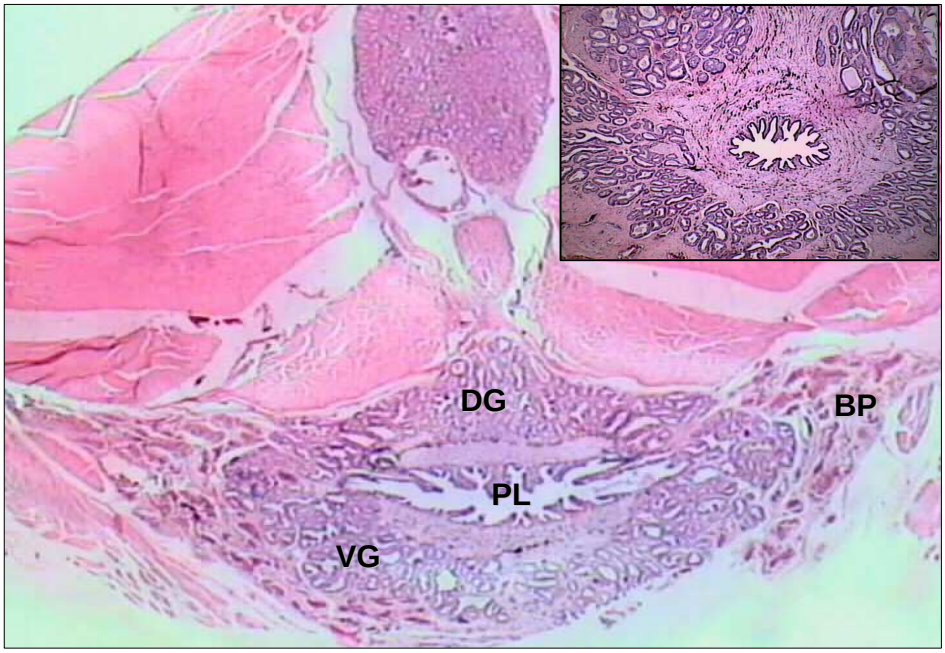

Figure 36. Proctodeum (PR) of a *Cordylus cataphractus* female, more posteriorly than in Figure 35. The dorsal (DG) and ventral (VG) glandular masses form a ring around the flattened proctodeal lumen (PL) (120x). Note the blood plexus (BP) lateral to each side of the proctodeum. The insert shows a semi-ring glandular arrangement in another individual (100x). Both are the same individuals as in Fig. 34.

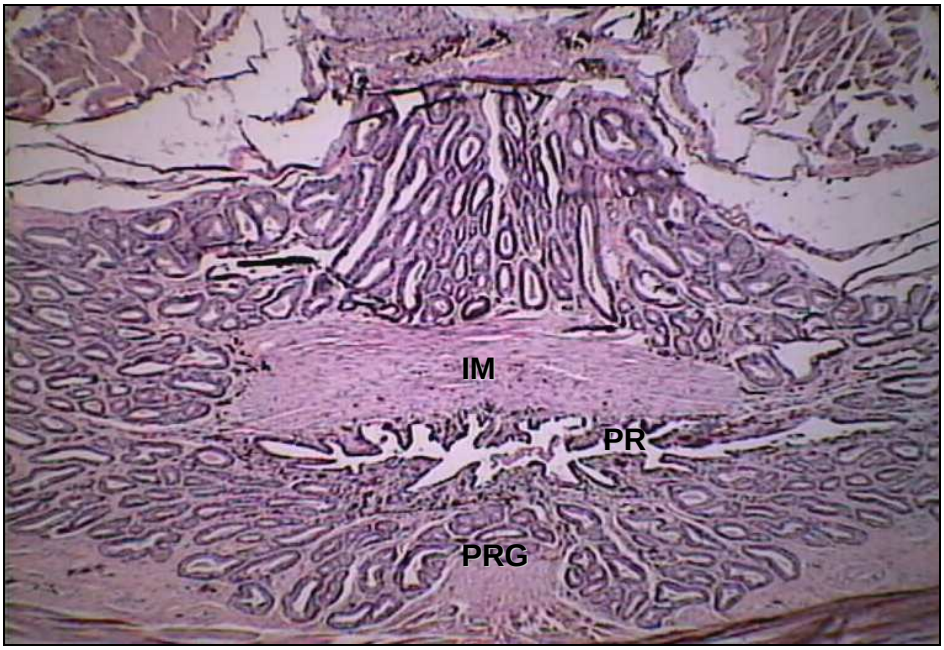

Figure 37. Proctodeum (PR) of a *Cordylus cataphractus* female. The muscle layer (IM) around the proctodeum (PR) becomes smaller ventrally as the proctodeum flattens and reduces dorsally as the proctodeal glands (PRG) form a ring around the proctodeum (100x).

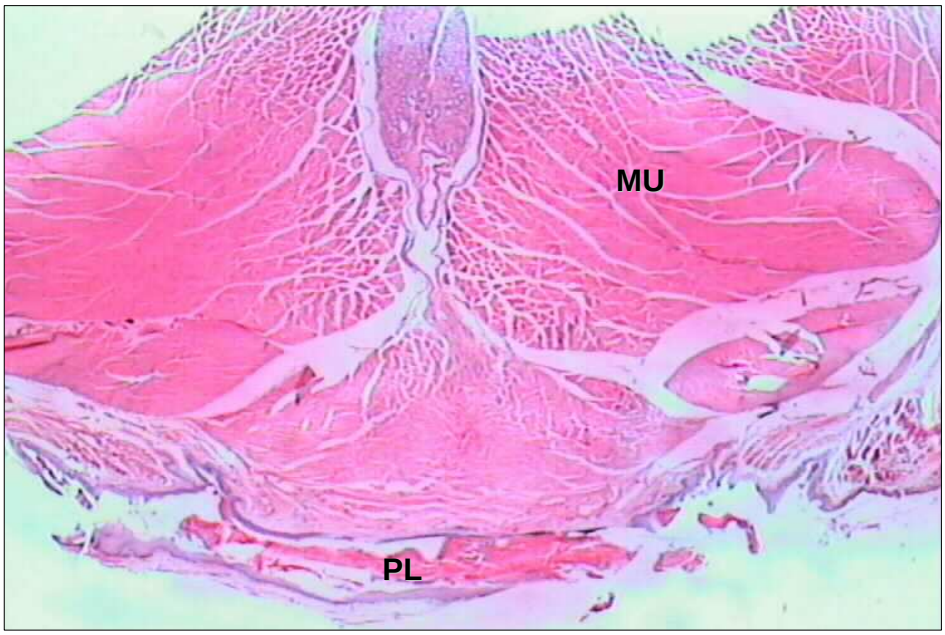

Figure 38. Proctodeum of a *Cordylus cataphractus* female. The flattened proctodeal lumen (PL) at the vent is lined with a thick stratified squamous epithelium (120x).

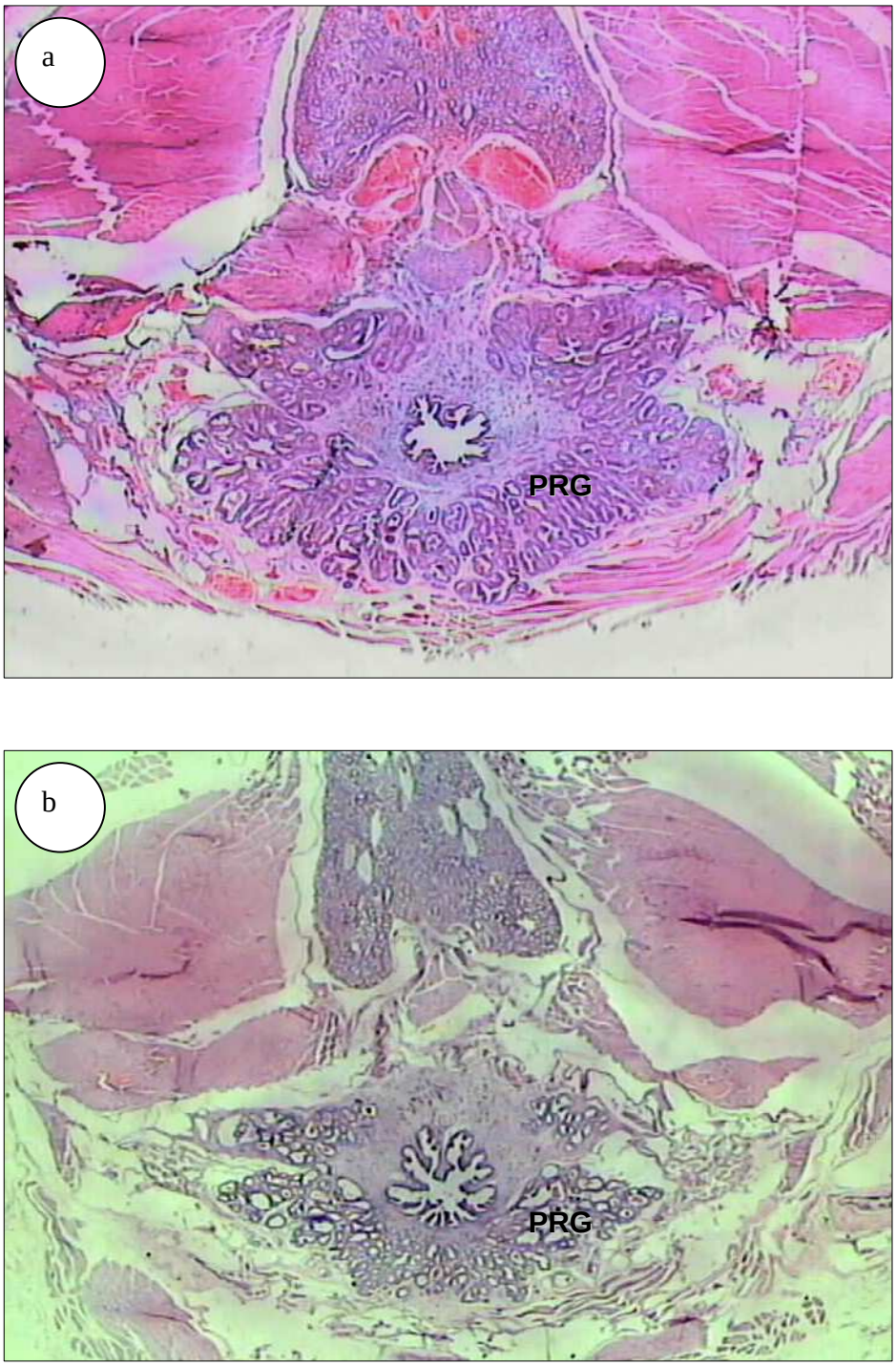

Figure 39. Dorsal and ventral clusters of the proctodeal glands (PRG) are present in both sexes of *Cordylus cataphractus*, but are more prominent in a) males than in b) females. Both magnified at 120x and stained with H & E, (although different batches).

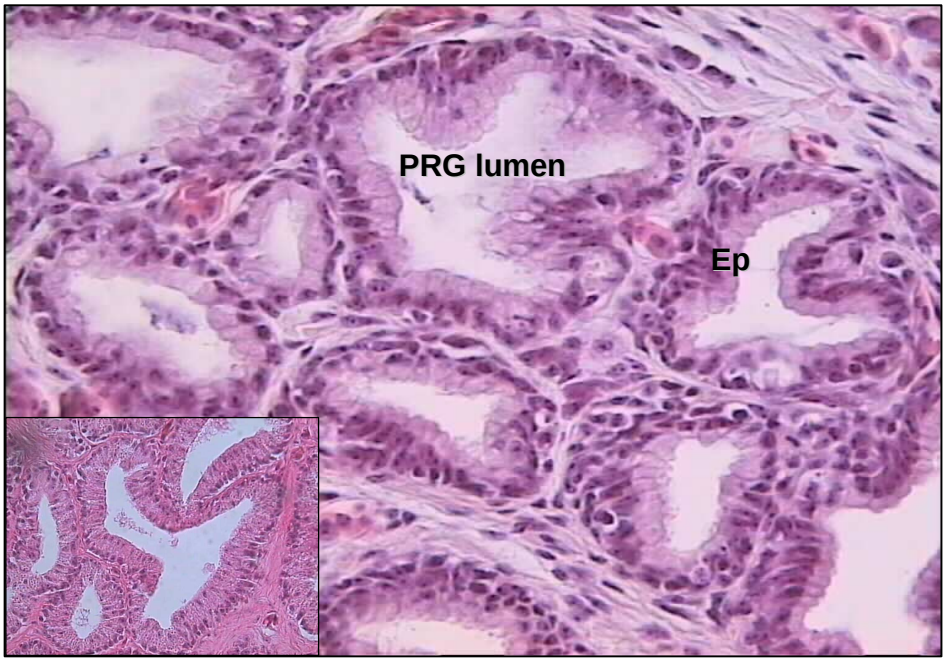

Figure 40. Proctodeal glands (PRG) of a *Cordylus cataphractus* female and male (insert). Note the large, irregularly sized lumina of the tubules. Ep = epithelium layer (both at 400x).

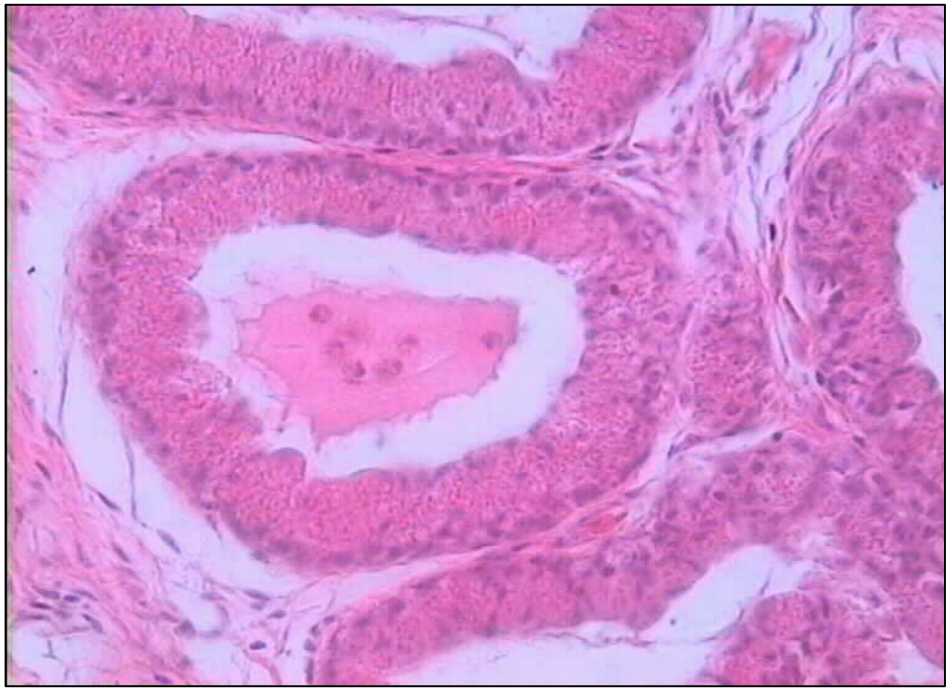

Figure 41. Proctodeal glands of a *Cordylus cataphractus* male, stained with H & E. Note the granular appearance of the epithelium (Ep) in dorsal gland (DG) tubule. The secretory material (SM) inside lumen appears uniform (1000x).

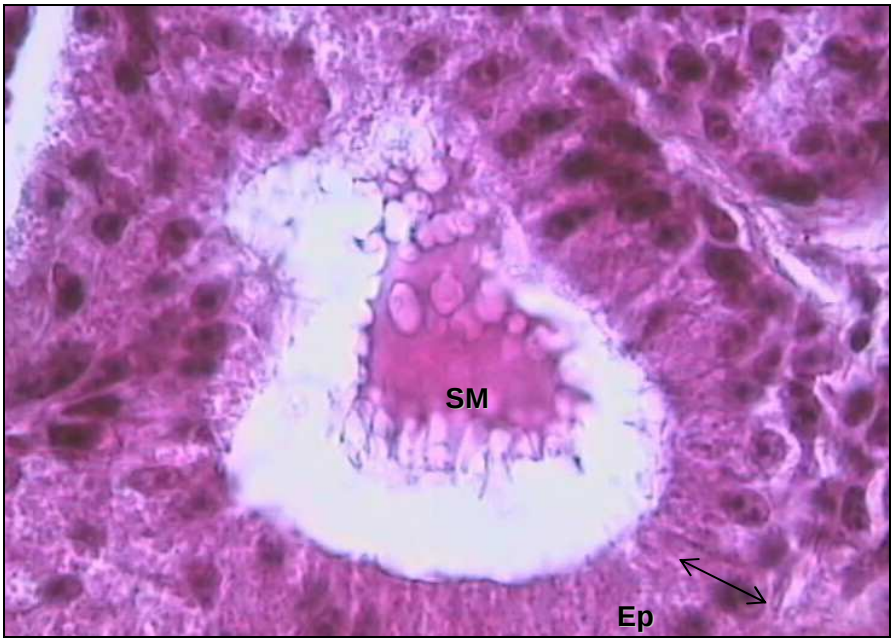

Figure 42. Secretory material (SM) found in proctodeal gland of a *Cordylus cataphractus* female, stained with Masson's Trichrome (1000x). Ep = Epithelium.

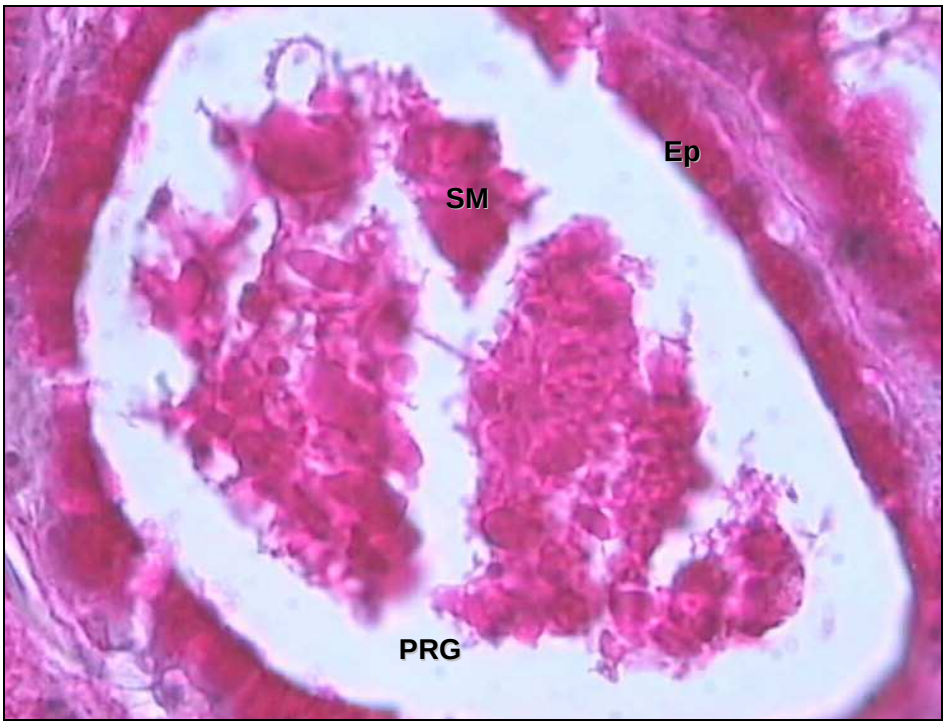

Figure 43. Secretory material (SM) found in proctodeal gland of a *Cordylus cataphractus* female at mid-gestation. Note that both the epithelium (Ep) and secretory material stain positively with PAS (1000x).

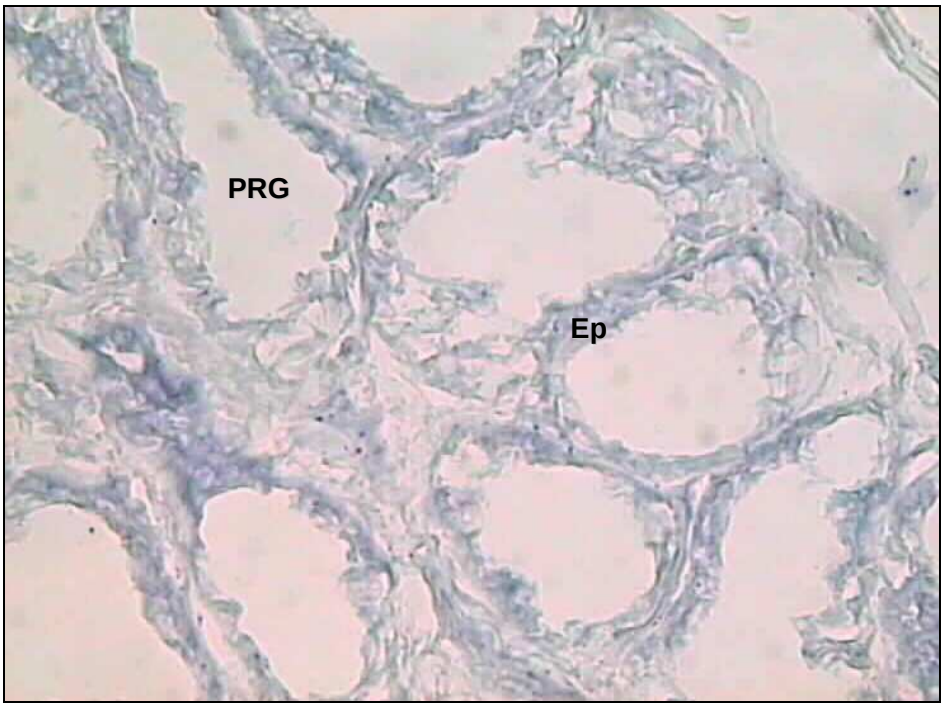

Figure 44. Proctodeal glands (PRG) of a *Cordylus cataphractus* female at mid gestation. Neither the epithelium nor secretory material stain positively with Sudan Black B (1000x).

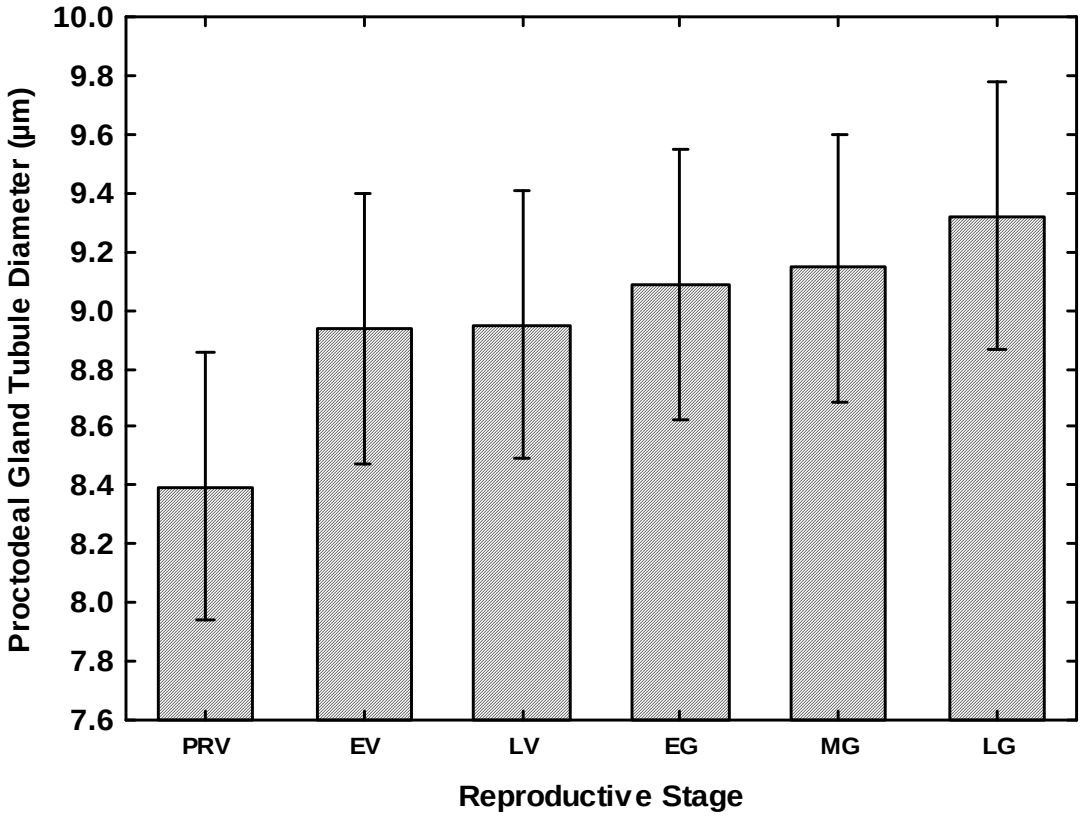

Figure 45. Variation in proctodeal gland tubule diameter (means ± 1 standard error) among female *C. cataphractus* in different reproductive stages. Stages are, PRV = Pre-vitellogenic; EV = Early vitellogenic; LV = Late vitellogenic; EG = Early gravid; MG = Mid-gravid; LG = Late gravid.

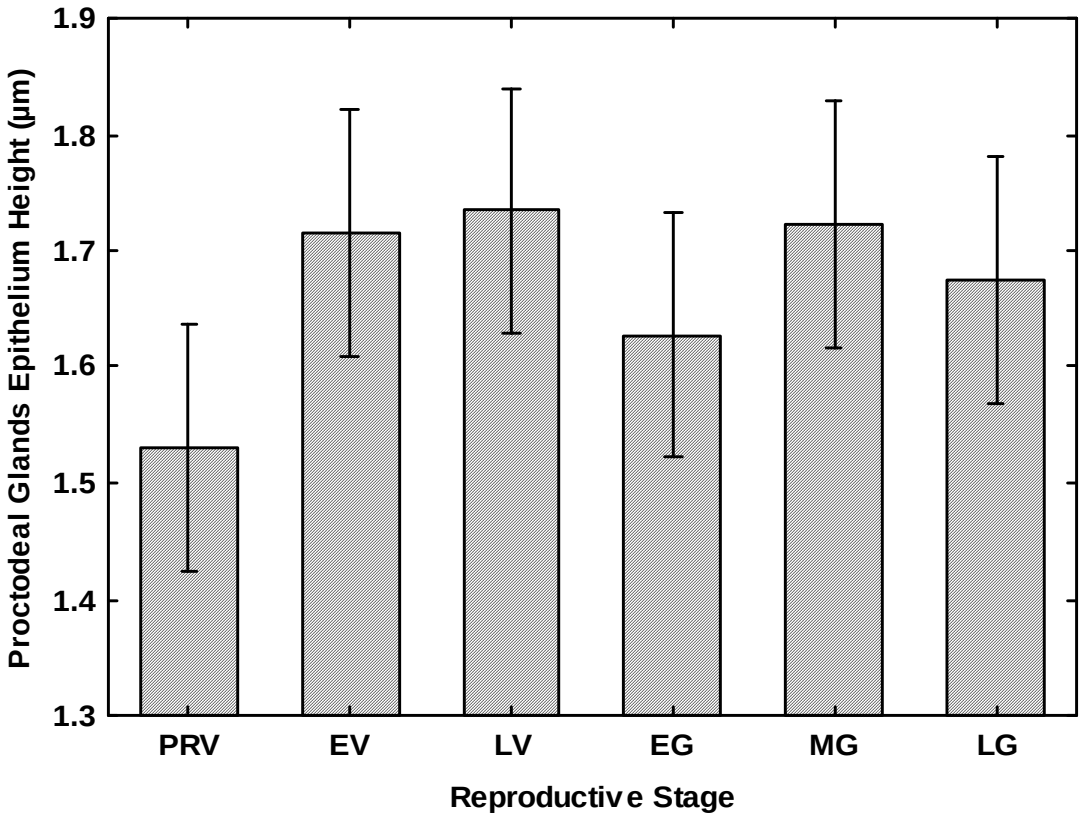

Figure 46. Variation in proctodeal gland epithelium height (means ± 1 standard error) among female *C. cataphractus* in different reproductive stages. Stages are, PRV = Pre-vitellogenic; EV = Early vitellogenic; LV = Late vitellogenic; EG = Early gravid; MG = Mid-gravid; LG = Late gravid.

– DISCUSSION –

1. *Structure of the proctodeal glands*

The gross anatomy of the proctodeum and its glands in *Cordylus cataphractus* generally agrees with that described in other lizards (Gadow, 1887; Trauth *et al.*, 1987; Withers & O'Shea, 1993). Proctodeal glands occur as prominent dorsal and ventral masses in both sexes of *C. cataphractus*, which are more distinct in males than in females. This is also the case in two congenerics studied previously, namely *C. cordylus* and *C. melanotus* (Mackay, 1993). Mackay (1993) stated that in *C. melanotus*, the dorsal clusters of the proctodeal glands in males extend further backwards than the ventral ones and in females he found the opposite. In both sexes of *C. cordylus*, the dorsal clusters of the proctodeal glands extended further backwards than the ventral clusters. Cooper & Trauth (1992) reported that in *Gerrhosaurus nigrolineatus* (closely related to *Cordylus*, as it belongs to the sister family of the Cordylidae, namely the Gerrhosauridae), the dorsal proctodeal glands appear more anterior to the ventral proctodeal glands. I found no definite pattern in *C. cataphractus* in this regard (see Chapter 2 for details), as too much variation was apparent. One must be cautious in interpreting differences noted by Cooper & Trauth (1992) and Mackay (1993) as they had small sample sizes and the extent of intraspecific variation is unknown.

Male lizards of the Gekkonidae, Iguanidae, Scincidae & Xanthusiidae have mixed glands, containing both mucous and serous cells, while females only have mucus-

secreting cells. According to Gabe & Saint Girons (1965) and Whiting (1969) mucous cells tend to be concentrated in the ventral and serous cells in the dorsal glands of Scincidae and Gekkonidae. Mackay (1993) found in both cordylid species studied, that the proctodeal glands contain mainly mucous cells, but that serous cells occur in some areas. Mucus-secreting cells predominate in *C. cataphractus*, but serous cells are also found in both ventral and dorsal clusters of the proctodeal glands in both sexes.

2. Seasonal variation in proctodeal glands

Proctodeal glands, in contrast to urodeal glands, show little or no seasonal variation in development or secretory activity in both sexes of *C. cataphractus*, conforming to the general pattern found in other lizards (Regamey, 1935; Cooper & Trauth, 1992; Mackay, 1993). None of the mentioned authors had proper samples to test for seasonal variation in proctodeal glands. Their findings on seasonal variation or the lack thereof must be treated with caution, especially in the light of the high between-individual variation noted in the present study. Secretion quantities are highly variable among *C. cataphractus* individuals of the same sex and time of year, but there was no evidence of sex related differences in the morphometric analyses. Mean diameters and epithelial heights measured in the proctodeal gland tubules do not differ significantly among females in different reproductive stages.

3. Possible functions of proctodeal glands

A few authors suggested that proctodeal glands may have a purely mechanical function in lizards. Lerebouillet (1851), for example, suggested that the secretions of the proctodeal glands serve to keep the cloaca moist, while Wöpke (1931) suggested that the secretions serve as substrate for genital products or serve as lubrication to facilitate mating. Gabe & Saint Girons (1965) mentioned that they may simply facilitate mating and further displace the sperm cells towards the female genitals.

Another possible function suggested in the literature, is that the glands are a potential source of pheromones (Whiting, 1969; Trauth *et al.*, 1987; Cooper & Trauth, 1992; Cooper, *et al.*, 1996). Trauth *et al*. (1987) for instance, suggested that the dorsal glands in *Eumeces laticeps* produce a species-identifying (to identify male sexual competitors) pheromone present in both sexes. They suggest that males of the above species can discriminate between chemical cloacal stimuli of conspecific and heterospecific males and between cloacal stimuli of conspecific and heterospecific females. Thus, the combined absence of the urodeal pheromone (if urodeal glands secrete a pheromone) and the presence of the species-identifying pheromone of the proctodeal glands in conspecifics might serve to identify male sexual competitors in Scincidae (Cooper *et al.*, 1986; Cooper *et al.*, 1987) and also in *Gerrhosaurus nigrolineatus* (Cooper & Trauth, 1992). The possible pheromonal function of the gland secretions may explain why the proctodeal glands are more conspicuous in males than females of these species.

Bull *et al.* (1999) noted that the skink, *Egernia striolata*, can discriminate between their own scats and other scats. This may suggest that the lizards respond to skin or cloacal gland secretion, for example, scats produced in the hind-gut could become coated with a secretion from cloacal glands as they pass during defecation. The response to scats in *E. striolata* from unfamiliar conspecifics was stronger than the response to novel scats from another species. According to Bull *et al*. (1999) scat piles have the potential to be a useful signal of territory ownership and they also supported the hypothesis that "scat piling" may have a function of signaling residency (i.e., a means of communication and maintenance of social structure within a population).

I propose that, in the case of proctodeal glands of *C. cataphractus* a lubrication function is not excluded - many mucous cells are present and the histochemical properties of secretions support this assumption. The secretory product is copious, acidophilic and rich in glycoproteins. If it indeed contributes to lubrication, it is probably related to a frequent event like defecation, as secretion was found in both sexes and no seasonal variation in secretory activity was observed. The possibility of pheromonal communication seems also very likely as a serous component is evident and glands are larger in males. If so, then urodeal gland secretion is again probably not related to a seasonal event such as eliciting courtship or attracting mates, but, for example, in territory marking, which probably occurs throughout the year. Further investigation is needed, particularly chemical analysis of secretions and behavioural manipulation, before any conclusive statements can be made on the role of the proctodeal glands in pheromonal social communication in this animal. I have stained for lipoidal substances in the secretions of a *C. cataphractus* female in mid-gestation, but found a negative Sudan

Black B reaction. It is possible that the glands have a dual function and that pheromonal substances are added seasonally without influencing secretion quantities markedly. A seasonal profile of possible lipoidal secretion would have been helpful in commenting on this possibility, and future investigations should take this direction.

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- APPENDIX 1 –

Details of references containing descriptions of general cloacal anatomy of lizards, including cloacal glands.

Publications	Species	Family	Sex	General Cloacal	Urodeal Glands		Proctodeal Glands		Possible
				Description	General description	Histo chemistry	General description	Histo chemistry	Function
Mackay (1993)	*Cordylus melanotus*	Cordylidae	3♀ 3♂	*	*		*		
	Cordylus cordylus	Cordylidae	4♂ 5♀	*					
Unterhössel (1902)	*Pachydactylus guttatus*	Gekkonidae		*					
Cooper & Trauth (1992)	*Gerrhosaurus nigrolineatus*	Gerrhosauridae	2♂ 2♀	*	*		*		Pheromonal
Forbes (1941)	*Scleroporus spinosus floridanus*	Iguanidae	38♂				*		
Disselhorst (1904)	*Lacerta agilis*	Lacertidae		*			*		
Gadow (1887)	*Lacerta ocellata*	Lacertidae		*	* "anal glands"				
	Lacerta viridis	Lacertidae		*					

Publications	Species	Family	Sex	General Cloacal	Urodeal Glands		Proctodeal Glands		Possible
				Description	General description	Histo chemistry	General description	Histo chemistry	Function
Ihle (1947)	*Lacerta viridis*	Lacertidae		*					
Regamey (1935)	*Lacerta agilis*	Lacertidae		*	*		*		Mechanical (lubrication)
Wöpke (1931)	*Lacerta agilis*	Lacertidae		*			*		Mechanical
Cooper & Gartska (1987)b	*Eumeces laticeps*	Scincidae			*		*		
Cooper, Gartska & Vitt (1986)	*Eumeces laticeps*	Scincidae	2♀		*	*			Pheromonal
Cooper & Vitt (1986a)	*Eumeces laticeps*	Scincidae							Pheromonal
Trauth, Cooper, Vitt & Perril (1987)	*Eumeces laticeps*	Scincidae	3♂ 5♀	*	*	*	*	*	Pheromonal (both glands)
Van der Merwe (1944)	*Acontias meleagris*	Scincidae		*			*		Mechanical
Hardy & Cole (1981)	*Cnemidophorus exsanguis*	Teiidae	10♀	*					
Gabe & Saint Girons (1965)	**different reptile species**			*	*	*	*	*	Mechanical
Whiting (1969)	**different reptile species**			*	*		*		

- APPENDIX 2 –

Detailed list of male *Cordylus cataphractus* specimens used in this study, with collection- and locality data. Specimens were accessioned into the Ellerman Collection of Stellenbosch University (refer to the lodging codes).

Reproductive status	**Date collected**	**Lodging code**	**SVL (mm)**	**Locality**	**Quarter Degree**	**Collector/s**
Early spematogenic (2)	24/10/96	USEC/H-3070	108.65	Dronkvleikop	3118CCCB	leF Mouton
Peak spematogenic (3)	24/10/96	USEC/H-3080	119.65	Dronkvleikop	3118CCCB	leF Mouton
Inactive stage (1)	25/10/96	USEC/H-3092	114.3	Dronkvleikop	3118CD	leF Mouton
Inactive stage (1)	04/12/96	USEC/H-3096	123.0	Puts	3118CD	leF Mouton
Inactive stage (1)	05/12/96	USEC/H-3099	115.34	Puts	3118CCCB	leF Mouton

Detailed list of female *Cordylus cataphractus* specimens used in this study, with collection- and locality data. Specimens were accessioned into the Ellerman Collection of Stellenbosch University (refer to the lodging codes).

Reproductive status	**Date collected**	**Lodging code**	**SVL (mm)**	**Locality**	**Quarter Degree**	**Collector/s**
Pre-vitellogenic	23/02/88	USEC/H-1693	101.45	Verlorenvlei	3218AD	NC Badenhorst
Pre-vitellogenic	02/04/97	USEC/H-3115	100.8	Klipfontein	3118DC	leF Mouton
Pre-vitellogenic	02/04/97	USEC/H-3138	100.03	Klipfontein	3118DC	leF Mouton
Pre-vitellogenic	02/04/97	USEC/H-3143	96.78	Klipfontein	3118DC	leF Mouton
Pre-vitellogenic	02/04/97	USEC/H-3152	100.4	Klipfontein	3118DC	leF Mouton
Pre-vitellogenic	02/04/97	USEC/H-3189	98.59	Klipfontein	3118DC	leF Mouton
Early vitellogenic	15/07/84	USEC/H-773	104.65	Hotwegkloof	3219CA	leF Mouton, D Mostert
Early vitellogenic	07/04/89	USEC/H-1790	101.52	Katbakkies, Groenfontein	3219DC	leF Mouton
Early vitellogenic	21/09/89	USEC/H-1806	103.12	Skietkop, Elands Bay	3218AD	NC Badenhorst

Early vitellogenic	27/05/93	USEC/H-2418	103.87	Witwater	3018AB	leF Mouton
Early vitellogenic	27/05/93	USEC/H-2423	103.63	Witwater	3018AC	leF Mouton
Late vitellogenic	24/10/96	USEC/H-3079	112.75	Dronkvleikop	3118CCCB	leF Mouton
Late vitellogenic	24/10/96	USEC/H-3085	110.58	Dronkvleikop	3118CCCB	leF Mouton
Late vitellogenic	24/10/96	USEC/H-3090	103.16	Dronkvleikop	3118CCCB	leF Mouton
Late vitellogenic	24/10/96	USEC/H-3093	107.86	Dronkvleikop	3118CCCB	leF Mouton
Late vitellogenic	24/10/96	USEC/H-3094	114.78	Dronkvleikop	3118CCCB	leF Mouton
Early vitellogenic	05/12/96	USEC/H-3100	103.88	Puts	3118CCCB	leF Mouton
Early gravid	05/12/96	USEC/H-3104	104.05	Puts	3118CCCB	leF Mouton
Early gravid	05/12/96	USEC/H-3107	109.50	Puts	3118CCCB	leF Mouton
Early gravid	05/12/96	USEC/H-3110	115.41	Puts	3118CCCB	leF Mouton
Early gravid	05/12/96	USEC/H-3112	106.35	Puts	3118CCCB	leF Mouton

Mid-gravid	08/12/83	USEC/H-403	110.90	Ribbokplaat	3218AD	leF Mouton, D Mostert
Mid-gravid	20/02/99	USEC/H-3477	96.10	Bergopklip area	3118CCCD	leF Mouton
Mid-gravid	20/02/99	USEC/H-3478	102.30	Bergopklip area	3118CCCD	leF Mouton
Mid-gravid	20/02/99	USEC/H-3481	105.64	Bergopklip area	3118CCCD	leF Mouton
Mid-gravid	20/02/99	USEC/H-3511	103.80	Bergopklip area	3118CCCD	leF Mouton
Mid-gravid	13/01/04	USEC/H-4502		Graafwater	3118DC	D du Toit, A Alblas
Late gravid	02/04/97	USEC/H-3129	103.9	Klipfontein	3118DC	leF Mouton
Late gravid	02/04/97	USEC/H-3132	109.4	Klipfontein	3118DC	leF Mouton
Late gravid	02/04/97	USEC/H-3134	101.00	Klipfontein	3118DC	leF Mouton
Late gravid	02/04/97	USEC/H-3149	114.0	Klipfontein	3118DC	leF Mouton
Late gravid	02/04/97	USEC/H-3150	108.98	Klipfontein	3118DC	leF Mouton
Late gravid	02/04/97	USEC/H-3158	111.17	Klipfontein	3118DC	leF Mouton
Mid-gravid (Macro)	20/02/99	USEC/H-3480	102.60	Bergopklip area	3118CCCD	leF Mouton